ANAEROBIC FERMENTATION OF RICE STRAW AND CHICKEN MANURE TO CARBOXYLIC ACIDS

A Dissertation

by

FRANK KWESI AGBOGBO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2005

Major Subject: Chemical Engineering

ANAEROBIC FERMENTATION OF RICE STRAW AND CHICKEN MANURE TO CARBOXYLIC ACIDS

A Dissertation

by

FRANK KWESI AGBOGBO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Chair of Committee, Mark T. Holtzapple
Committee Members, Richard R. Davison
Mahmoud M. El-Halwagi
Cady Engler
Head of Department, Kenneth R. Hall

December 2005

Major Subject: Chemical Engineering

ABSTRACT

Anaerobic Fermentation of Rice Straw and Chicken Manure to Carboxylic Acids. (December 2005)

Frank Kwesi Agbogbo, B.Sc., University of Science and Technology, Kumasi, Ghana

Chair of Advisory Committee: Dr. Mark T. Holtzapple

In this work, 80% lime-treated rice straw and 20% lime-treated chicken manure were used as substrates in rotary fermentors. Countercurrent fermentation was performed at various volatile solid loading rates (VSLR) and liquid residence times (LRT). The highest acid productivity of 1.69 g/(L·d) was at a total acid concentration of 32.4 g/L. The highest conversion and yield were 0.692 g VS digested/g VS fed and 0.29 g total acids/g VS fed, respectively. The continuum particle distribution model (CPDM) was used to predict product concentrations at various VSLR and LRT. CPDM predicted the experimental total acid concentration and conversion at an average error of 6.41% and 6.55%, respectively.

A fixed-bed fermentation system was designed to perform pretreatment and fermentation in the same unit. High product concentrations (~48 g/L) as well as high conversions (0.741 g VS digested/g VS fed, F4, Train B) were obtained from the same fermentor. CPDM was extended to predict product concentrations in the fixed-bed fermentation system. The model gave a good estimate of the product concentrations and retention time.

After biomass fermentation, the residue can be combusted to generate heat. For pretreatment purposes, the use of ash can replace lime. A study was performed using ash as a potential pretreatment agent. Ash from raw poplar wood was effective in pretreating poplar wood; however, ash from bagasse fermentation residues was not useful in pretreating bagasse.

Previous modeling studies indicate that a conversion of 95% could be achieved with bagasse using countercurrent fermentation. Because lignin constitutes 13% of the dry weight of bagasse, this means lignin would have to be digested to obtain a conversion of 95%. Experiments on the fermentation of enzymatically liberated lignin from both poplar wood and bagasse do not show that solubilized lignin was fermented to organic acids by using a mixed culture of marine microorganisms.

Two buffer systems (ammonium bicarbonate and calcium carbonate) were used to compare product concentrations of carboxylic acid fermentations using office paper and chicken manure. It has been demonstrated that the total product concentration using ammonium bicarbonate is almost double the product concentration using calcium carbonate.

ACKNOWLEDGEMENTS

I would like to thank my research advisor Dr. Mark T. Holtzapple for his guidance, supervision, and support for this research. Our conversations and brain storming sessions have generated projects we call the ‘wild cats.’ These ‘wild cats’ were investigated at the laboratory, some were successful, others were not. I would also like to thank Dr. Richard R. Davison for his interest in our research and for visiting our laboratory frequently to see if I got any new data. I had very useful discussions and learnt a lot from him. I would like to thank Dr. Mahmoud M. El-Halwagi who taught me process integration and made me always think of what will happen to the entire process when making changes in even a small part of the MixAlco process. His wise counsel has been of great help. I want to thank Dr. Cady Engler who taught me bioreactor design and has been helpful in providing useful insights and suggestions for my research. I want to thank Dr. Theresa Good for recommending me for admission and Dr. Christine Grant for getting me interested in environmentally related research.

I also like to thank the engineering technicians, Randy Marek and Nathan Okonski, for their assistance in making the fermentors and teaching me how to use the machines. I am indebted to my past group members Cateryna Aiello-Mazzarri, Sehoon Kim, Piyarat Thanakoses, Wen-Ning Chan, Guillermo Coward-Kelly, Cesar Granda, and Xu Li for their assistance. Thanks to my present group members Zhihong Fu, Jonathan O’Dwyer, Li Zhu, Maxine Jones, Rocio Sierra, and Stanley Coleman.

Finally, I like to thank the student workers who helped me to do the fermentations as well as the great secretaries at the Department of Chemical Engineering who were always ready to help.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I
INTRODUCTION

1.1 Biomass as a Sustainable Fuel and Chemical Source

Increasing energy demand and decreasing oil reserves make it necessary to find alternative sources of energy. Cellulosic materials are attractive as a sustainable source of fuels and chemicals because of their plentiful supply and relatively low cost. The technological impediment to the widespread use of this resource is the recalcitrance of the biomass.

Agricultural production generates wastes (e.g., animal manure and crop residues), which have disposal costs as well as adverse environmental impacts. The use of agricultural residues for fuels is not only beneficial as a sustainable energy source, but solves disposal problems and reduces greenhouse gases (Kheshgi *et al*., 2000). The three major components of waste biomass from agricultural residues are cellulose, hemicellulose, and lignin.

Cellulose is the most abundant component of biomass and is found almost exclusively in plant cell walls (Lynd *et al*., 2002). Despite the differences in composition and anatomical structure of cell walls, the cellulose content is typically in the range of 35 to 50% of plant dry weight. Cellulose fibers are usually buried in a matrix of other structural biopolymers, hemicellulose and lignin, which comprise 20 to 35% and 5 to 30% of plant dry weight, respectively (Lynd *et al*., 1999). These matrix interactions are the dominant structural feature limiting the rate and the extent of utilization of untreated biomass (Lynd *et al*., 2002).

Carboxylic acids (C2−C7) are produced from anaerobic fermentation. Because they have a high market value, these acids can be recovered and sold. Alternatively, they can be converted to methane (biogas) or chemicals (e.g., ketones, aldehydes, and alcohols). Obligately anaerobic bacteria arose early on earth and have adapted to

---

This dissertation follows the style and format of Bioresource Technology.

energy-stressed conditions to grow efficiently on a wide variety of substrates. Theoretical anaerobic fermentation yields of combustible energy are 33% for H_2 production, 85% for methane, and nearly 100% for acetic or butyric acids (Zeikus, 1980). This project was performed at mesophilic conditions (40^{o}C) using a mixed culture of marine microorganisms.

1.2 Components of Cellulosic Biomass

Cellulose is a linear condensation polymer consisting of D-anhydroglucopyranose joined together by β-1,4-glycosidic bonds (Figure 1-1). The glucosidic linkage acts as a functional group, and this along with the hydroxyl groups mainly determine the chemical properties of cellulose. The cellulose degree of polymerization (DP) ranges from 500 to 15000 (Holtzapple, 1993). Coupling of adjacent cellulose molecules by hydrogen bonds and van der Waal's forces result in parallel alignment and crystalline structure (Zhang and Lynd 2004). The less ordered region is called the amorphous region. The disordered region allows easier disintegration of cellulose by hydrolysis compared to the crystalline region. Individual cellulose molecules are linked together to form elementary fibrils, which are aggregated into long slender bundles called microfibrils.

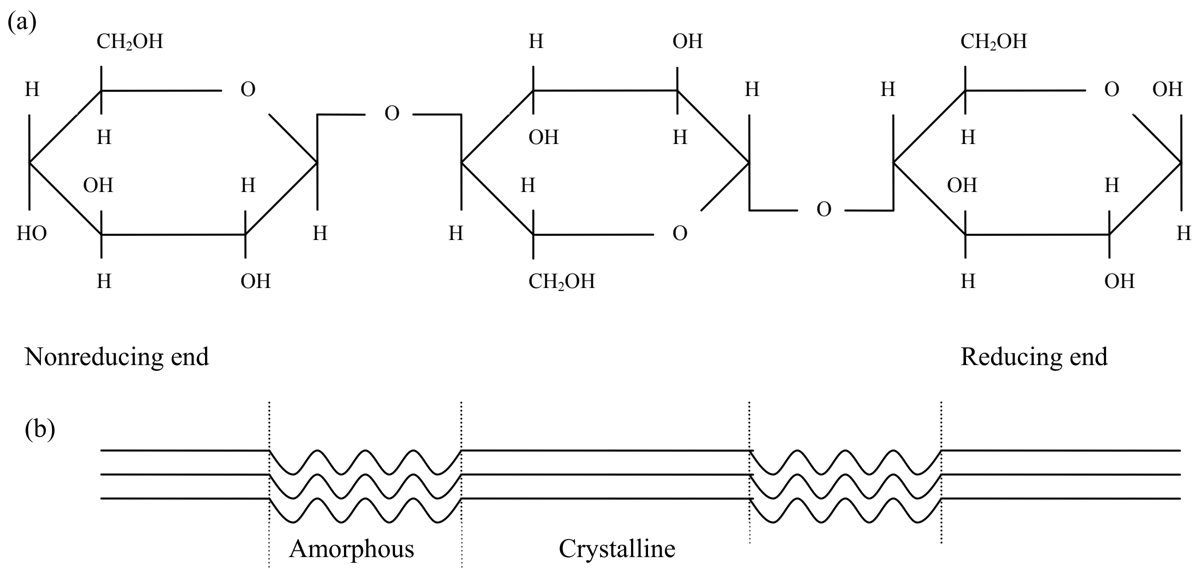
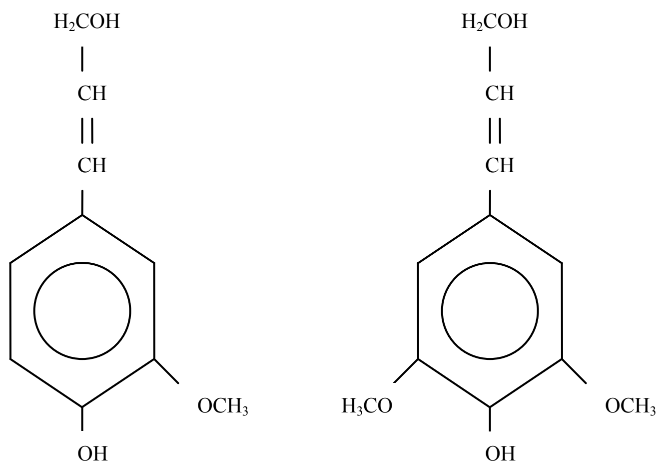


Figure 1-1. (a) Conformational form of cellulose (b) Cellulose microfibril.

Hemicellulose polymers are shorter than cellulose polymers with degree of polymerization of 50–200. Unlike cellulose, which is a linear polymer without side-groups, hemicellulose polymers are Y branched and most have attached side-groups. The role of hemicellulose is to provide a linkage between cellulose and lignin. Hemicellulose monomers consist of three hexoses (glucose, galactose, and mannose) and two pentoses (xylose and arabinose). Some of the sugars (glucose, mannose, and xylose) are acetylated. Both α (1,2; 1,3; 1,6) and β (1,2; 1,3; 1,4; 1,5; 1,6) sugars appear in hemicellulose, although the β forms are more common (Holtzapple, 1993).

Lignin is a three dimensional phenyl-propane polymer with phenylpropane units held together by ether and carbon-carbon bonds (Fan *et al*, 1987). It has a high molecular weight 5000 (DP about 25) and is amorphous in nature. Lignin gives structural rigidity and its hydrophobic nature prevents water loss from plant vascular systems. It is comprised of *trans*-coniferyl, *trans*-sinapyl, and *trans*-*p*-coumaryl alcohol monomers (Figure 1-2) and acts as the ‘net’ holding the fibrous structure together (Holtzapple, 1993).



Coniferyl alcohol   Sinapyl alcohol   *p*-Coumaryl alcohol

Figure 1-2. Lignin monomers.

The ultrastructural organization of the cell wall components (cellulose, hemicellulose, and lignin) is shown in Figure 1-3. The cellulose elementary fibril is surrounded by hemicellulose and lignin to prevent cellulose hydrolysis. Glucose production from cellulose is achieved by adding water molecules, and therefore the linkage is broken by hydrolysis. However, because cellulosic biomass is recalcitrant to hydrolysis due to its structural features, pretreatment is required to alter these features and make the biomass more digestible.

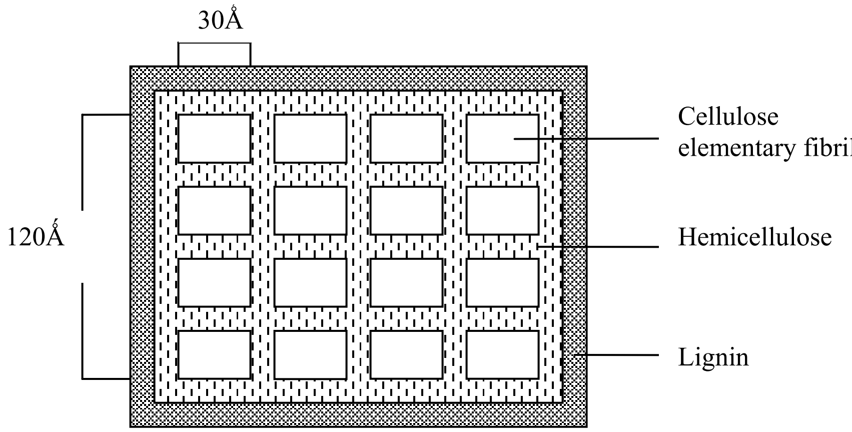


Figure 1-3. Ultrastructural organization of cell wall components (Fan *et al*., 1987).

1.3 Biomass Pretreatment

The rate and extent of utilization of lignocellulosic biomass is improved by pretreatment. Pretreatments are classified as mechanical, physical, chemical, or biological (Fan *et al*., 1987). Cellulose crystallinity, accessible surface area, protection by lignin, and cellulose sheathing by hemicellulose all contribute to biomass resistance (Mosier *et al*., 2004). Chemical pretreatments are simple and effective; various acids, alkalis, gases, alcohols, oxidizing, and reducing agents are used. Many alkalis are inexpensive and they cause less cellulose degradation and therefore are preferred chemicals. Lime pretreatment technology has been studied on various biomass sources such as switchgrass, corn stover, and wood (Chang *et al*., 1997 and 1998, Kaar and Holtzapple, 2000). Lime has the following advantages: it is inexpensive, safe to handle, and can be simply recovered (Chang *et al*., 1998).

1.4 Lime Pretreatment

Two types of lime treatment have been developed in our laboratory, short term and long term. Short-term lime pretreatment involves boiling the biomass with a lime loading of 0.1 g $Ca(OH)_2$/g dry biomass at temperatures of 85−135°C for 1−3 hours

(Chang *et al.*, 1997,1998). This removes approximately a third of the lignin and all the acetyl groups from hemicellulose (Chang and Holtzapple, 2000).

Chang *et al*. (2001) showed that oxidative lime pretreatment could be used to pretreat high-lignin biomass. Long-term pretreatment involves using lime loading at lower temperatures (40−55^{o}C) for 4−6 weeks in the presence of air. Kim (2004) showed that long-term pretreatment removes about half of the lignin and all the acetyl groups in corn stover. Carbohydrates in the presence of alkali and oxygen undergo both oxidation and alkaline degradation to produce a complex mixture of products (Kim, 2004). The endwise depolymerization reaction is a β-elimination that begins at the reducing end of the molecule and proceeds along the chain liberating saccharinate molecules (Lai, 2001).

In the case of manure, lime treatment leads to the saponification of fats making them more digestible (Thanakoses, 2002). Holtzapple *et al*. (1999) have shown that an optimum fermentor feed contains about 80:20 ratio of energy source (lignocellulose) to manure. This ratio ensures the optimum C/N ratio necessary for fermentation.

1.5 Rice Straw Conversion Technologies

Currently, rice straw is either burned in open fields or incorporated into the soil. Increasing environmental concerns and government legislation call for a decrease in the quantity of rice straw burned (California Rice Commission's Library on Rice Straw Utilization). Incorporating rice straw into soil increases foliar disease, reduces crop yield, degrades soil conditions, and produces methane, a greenhouse gas (Chidthaisong and Watanabe, 1997). Therefore, a low-cost technology to convert these wastes into useful fuels (Lynd *et al*., 2002) and chemicals is valuable. Significant potential benefits result from fuel derived from cellulosic biomass, a renewable nonfood feedstock (Lynd *et al*., 1991).

Rice straw contains 33% cellulose, 26% hemicellulose, and 18% lignin (Holtzapple, 1993). The conversion of rice straw to ethanol has been studied using simultaneous saccharification and fermentation (SSF) (Chadha *et al.*, 1995) where lignocellulose is simultaneously hydrolyzed to sugars and fermented to ethanol. Unfortunately, this process requires expensive enzymes and sterile operating conditions, both of which contribute heavily to production costs. Zhang and Zhang (1999) studied biogasification of rice straw to produce biogas (CH_4 (50%)); however, methane is a low-value product.

1.6 The MixAlco Process

An alternative to SSF is the MixAlco process, which converts biodegradable materials into mixed alcohols (Holtzapple *et al.*, 1999) (Figure 1-4). The MixAlco process involves anaerobic fermentation of biomass using a mixed culture of microorganisms to produce carboxylic acids (volatile fatty acids).

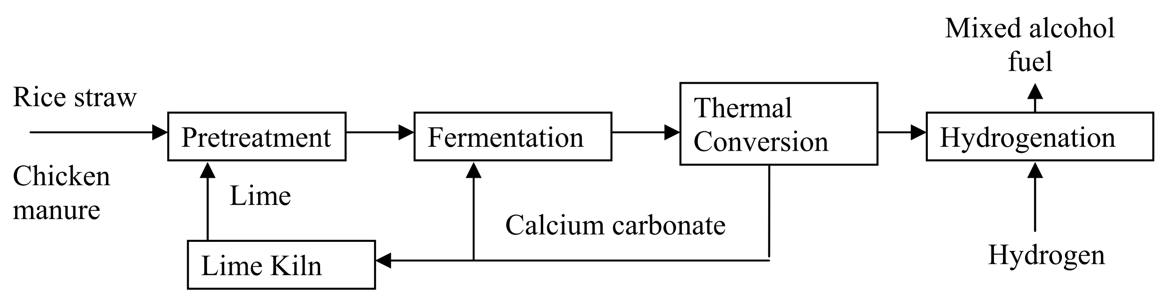


Figure 1-4. MixAlco process.

The first step in the MixAlco process involves lime treatment to render the biomass more digestible (Chang and Holtzapple, 2000, Fan *et al.*, 1982). The biomass is fermented to carboxylic acids and converted to carboxylate salts in the presence of a $CaCO_3$ buffer. These carboxylate salts can be concentrated, followed by "acid springing" to produce the corresponding organic acids (Chan and Holtzapple, 2003). Alternatively, the carboxylate salts can be converted to ketones and further hydrogenated to alcohol fuels. The MixAlco process has many benefits, such as no sterility requirement, adaptability to many feedstocks, and no enzyme addition. Rice straw has not been previously investigated as a feedstock for the MixAlco process.

Anaerobic fermentation utilizes microorganisms (bacteria, protozoa, and fungi) that produce enzymes in solution or on cell membranes that hydrolyze biomass. Such microbial systems exist in the rumen, swamps, compost, and marine ecosystem. Similar products are obtained from biomass using different sources of inocula (Thankoses, 2002, Aiello-Mazzarri, 2002); the main difference is the total acid concentration at the same volatile solid loading rate and liquid residence time.

The major pathway for lignocellulose fermentation in the rumen is given in Figure 1-5 (Prins, 1977). Cellulose and hemicellulose are hydrolyzed to produce sugar monomers with further reactions producing acetate, propionate, and butyrate as major products. However, excess reducing power (H_2) is generated, which can be used by methanogens to produce methane. Because osmotic stress is the ultimate barrier to achieving high acid concentrations, inocula from marine environments have salt-resistant microorganisms that strengthen the tolerance to high salt concentrations (Li, 2002).

Carboxylic acids are intermediates in the fermentation of biomass to methane (Datta, (1981), Fukuzaki *et al*., (1990)). In the MixAlco process, methane formation from carboxylic acids is inhibited using methane analogs, such as iodoform and bromoform, as inhibitors (Bauchop, 1967). This inhibition eliminates a potential hydrogen sink and instead is used to produce higher carboxylic acids, such as propionate and butyrate (Russell and Martin, 1984; Latham and Wolin, 1977). Other compounds such as monensin and pyromellitic diimide (Martin and Macy, 1985), coenzyme M analog 2-bromoethanesulfonic acid (2-BES) (Sauer and Teather, 1987), and more recently 2-nitro propanol (Anderson *et al*., 2003, 2004) has been found to inhibit methanogenesis.

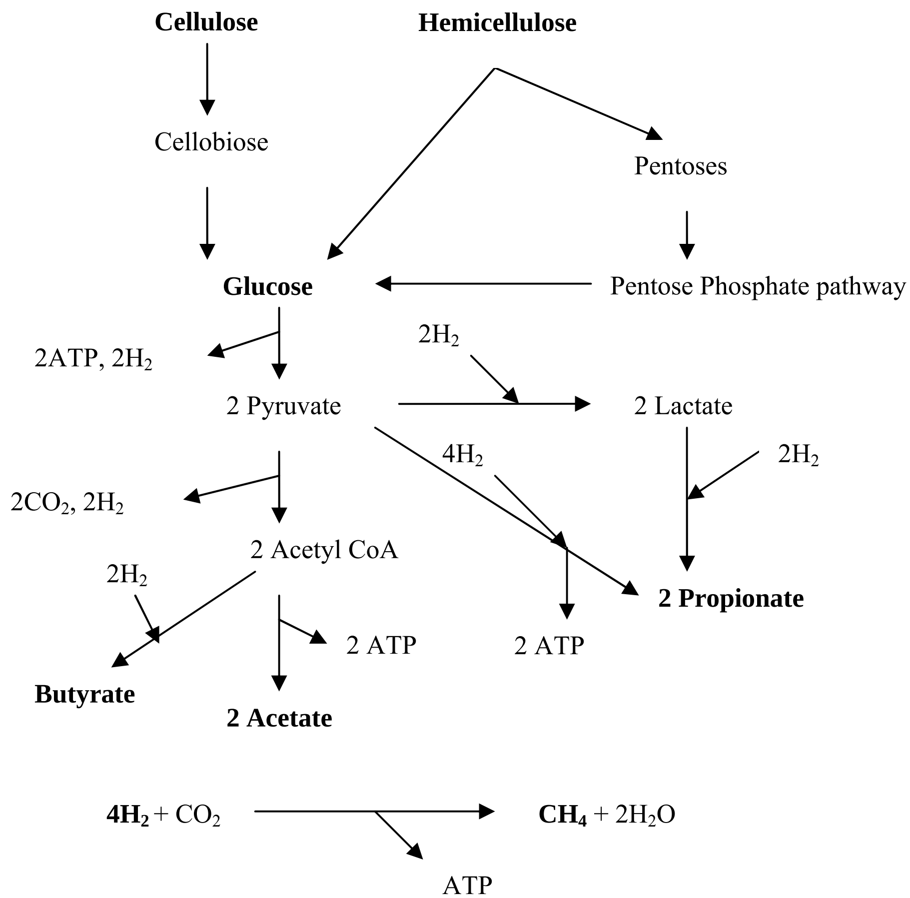


Figure 1-5. Major fermentation pathways in the rumen.

Biomass contains volatile solids (VS) and ash (Figure 1-6). When the biomass is digested, volatile solids convert to gaseous and liquid products, plus solid residues. The gaseous products are principally methane and carbon dioxide; the liquid products are carboxylate salts, extracellular proteins, and energy storage polysaccharides (Ross, 1998); and the solid residue contains ash and undigested VS.

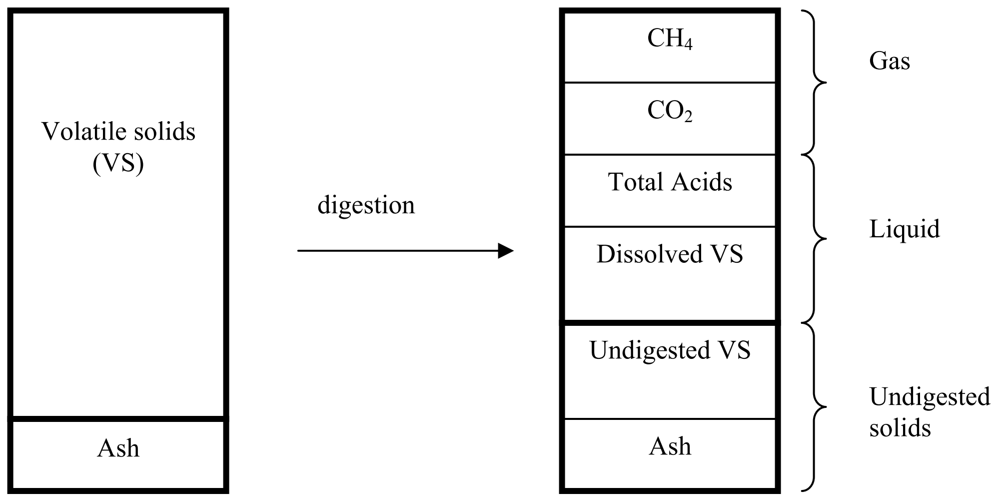


Figure 1-6. The digestion of biomass.

1.7 Countercurrent Fermentation and CPDM

To achieve high substrate conversions and product concentrations, countercurrent fermentation is used (Figure 1-7). Fresh biomass is added to the fermentor containing the highest carboxylate concentration while fresh water is added to the fermentor with the most digested biomass. This countercurrent flow arrangement addresses two issues: (1) biomass becomes more recalcitrant as the reactive components are digested, and (2) carboxylate salt products are extremely inhibitory (Ross and Holtzapple, 2001). Countercurrent fermentation was performed on a mixture of rice straw and chicken manure at various volatile solid loading rates (VSLR) and liquid residence times (LRT).

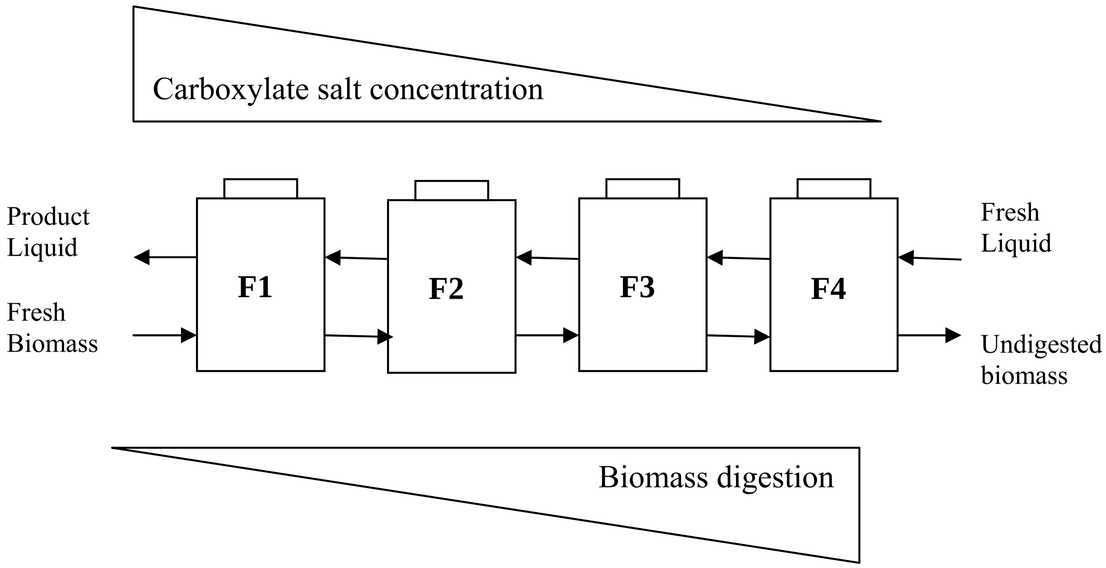


Figure 1-7. Four-stage countercurrent fermentation. (F1: Fermentor 1, F2: Fermentor 2, F3: Fermentor 3, and F4: Fermentor 4).

Countercurrent fermentation requires an extended time to reach steady state (~4 months). Because of the long residence time, it would be very time-consuming and cost-ineffective to explore a wide variety of operating conditions. To overcome this, Loescher (1996) developed the Continuum Particle Distribution Model (CPDM), a mathematical model that predicts the total acid concentration and substrate conversion using batch fermentation data. CPDM can save considerable time in determining the optimum operating conditions. The conversion penalty (South and Lynd, 1994) was used in the CPDM to account for the fact that biomass reactivity decreases at longer residence times because easy-to-digest portions react first. Data from the rice straw and chicken manure fermentation were used to verify the applicability of the CPDM results.

1.8 Fixed-bed Fermentation

The difficulty of transporting solids from one fermentor to another is a major drawback to applying countercurrent fermentation to industry. In the traditional approach to the MixAlco process, pretreatment and fermentation are separate unit operations. It is possible to combine these unit operations into a single step. In this work, a new fermentation system was designed to handle both pretreatment and fermentation as a single unit. In the new design, liquids were separated from solids by gravity, and transferred to the next fermentor as shown in Figure 1-8.

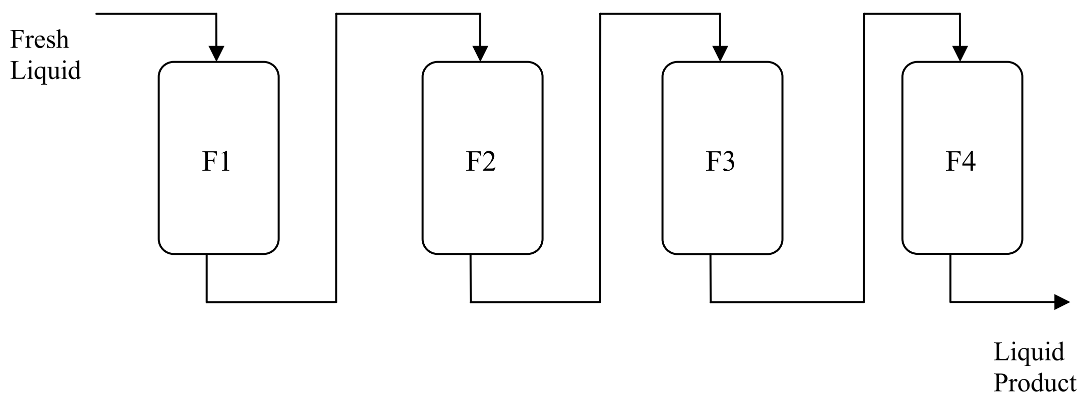


Figure 1-8. Liquid transfer in fixed-bed fermentor.

Because these agricultural residues are harvested annually or biannually, the new fermentation could be performed to digest the entire biomass over an extended period. Because this is a non-sterile system, the laboratory-scale fermentation columns may be built from inexpensive materials, such as PVC pipes. Rice straw and chicken manure were used as substrates in the fixed-bed fermentors. Different liquid transfer rates were studied in this fermentation system and CPDM was used to model product concentrations.

1.9 Lignin Degradation

Lignin has subunit structures consisting of nonrepetitive phenolpropane units randomly linked by various C—C and ether bonds, as well as intimate association and chemical cross-linking with the carbohydrate fraction of the plant cell wall. Lignin is difficult to degrade enzymatically and its presence with plant tissue significantly impedes cellulose degradation (Jeffries, 1990). Except for a few lignin-solubilizing and degrading actinomycetes (Crawford *et al*., 1983; McCarty, 1987; Ramachandra *et al*., 1988), lignin degradation is largely the domain of aerobic fungi. Brune *et al.,* (1995) have shown that lignin-derived aromatic ring cleavage by termite gut microbiota depends on oxygen.

Previous modeling studies indicate that a conversion of 95% could be achieved with bagasse using countercurrent fermentation (Thanakoses *et al*., 2003). Because lignin constitutes 13% of the dry weight of bagasse (Holtzapple, 1993), this means lignin would have to be degraded to obtain a conversion of 95%. The question that arises from this result is 'Is lignin digested by the microorganisms to produce organic acids or does it form part of the dissolved volatile solids in the liquid phase as shown in Figure 1-6?' To answer this question, an experiment was conducted where lignin was enzymatically isolated from biomass in a manner that minimizes chemical changes in lignin. Poplar wood or bagasse was used for this study. This enzymatically liberated lignin was subjected to air-lime pretreatment. Both soluble and insoluble residues were fed to a mixed-acid fermentation to determine if carboxylic acids could be made from the lignin residues.

1.10 Buffer

The buffer for the MixAlco process has traditionally been $CaCO_3$. Calcium carbonate is inexpensive and because it can be converted to lime for pretreatment purposes, calcium carbonate is a good choice. In feeding organic salts to animals, ammonium salts can he fed at higher dosages compared to other anionic salts (Oba and

Allen, 2002; Hutjens; Otterby *et al*., 1990). Amino carboxylate salts provide both a carbon and nitrogen source when used as animal feed. Other benefits of ammonium salts are inhibition of methanogenesis (Kayhanian, 1999; Parkin *et al*., 1980) and prevention of scale formation in downstream heat exchangers. Another benefit of NH_4HCO_3 fermentation is its effect as a supplemental nitrogen source for microorganisms. NH_4HCO_3 was used as a new buffer system in biomass fermentation at mesophilic conditions. To prevent interference effects of residual calcium from lime pretreatment, paper and chicken manure were used for this study because neither requires pretreatment to obtain high reactivity.

The reactions of the acids with the salts are given by Equations 1-1 and 1-2.

$$2CH_3(CH_2)_xCOOH + CaCO_3 \rightarrow Ca(CH_3(CH_2)_xCOO)_2 + H_2O + CO_2 \quad (1\text{-}1)$$

$$CH_3(CH_2)_xCOOH + NH_4HCO_3 \rightarrow NH_4CH_3(CH_2)_xCOO + H_2O + CO_2 \quad (1\text{-}2)$$

where x = 0, 1, 2, 3, 4, 5

The effect of increasing acid concentrations on pH is shown in Figure 1-9. The effect of pH on acid dissociation is given by the Henderson-Hasselbalch equation (Equation 1-3). The pH and pKa of the buffer determines the fraction of [A^-] and [HA] in solution. The pKa for ammonium bicarbonate and calcium carbonate are different and therefore, they buffer at a different pH.

$$\mathrm{pH} = \mathrm{pK_a} + \log_{10}\frac{[\mathrm{A^-}]}{[\mathrm{HA}]} \quad (1\text{-}3)$$

where [HA] = concentration of undissociated acid (mol/L)

[A^-] = concentration of acid anion (mol/L)

pK_a = acid dissociation constant

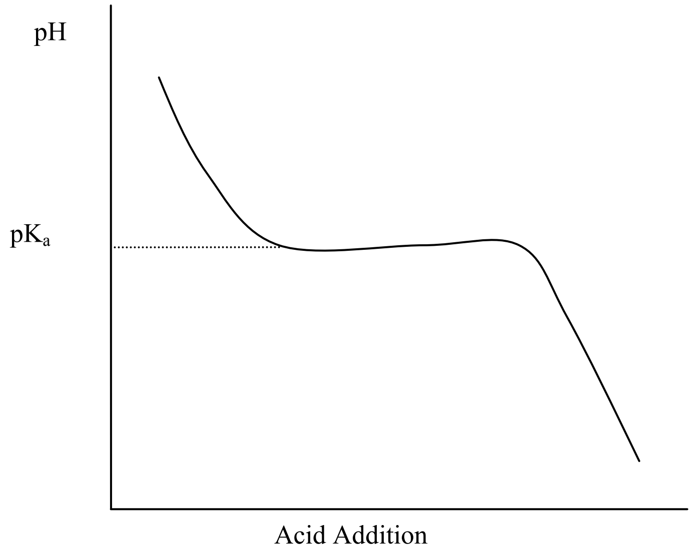


Figure 1-9. Titration curve for organic acids.

1.11 Ash Pretreatment

After biomass fermentation, the residue can be combusted to generate heat. The ash has disposal problems and the leachate (if ash is buried in landfills) could affect ground water. Biomass ash contains various oxides such as CaO, Na_2O, K_2O (Hisashi *et al*., 2003). Potentially, these oxides can be used to pretreat biomass. The use of ash for pretreatment may replace lime for pretreatment. Moreover, ash has been shown to improve biomass hydrolyzate fermentation by providing nutrients (Hisashi *et al*., 2003). In this study, poplar wood ash and bagasse ash were used.

CHAPTER II
MATERIALS AND METHODS

2.1 Substrates

Rice straw (RS) was obtained from Lee Tarpley of the Texas A&M Agricultural Research and Extension Center, Beaumont, Texas. The substrate was milled in a Thomas Wiley Laboratory mill and passed though a 2-mm screen. Chicken manure (CM) was obtained from the Poultry Science Center, Texas A&M University, College Station, Texas. The manure was air dried.

Short-term pretreatment (Appendix A) was performed on RS and CM for countercurrent fermentation. Pretreated rice straw (80%) and pretreated chicken manure (20%) (dry weight basis) were used as substrates in this research. The average moisture content of the pretreated RS was 0.028 g water/g raw RS, the average ash content was 0.274 g ash/g dry RS, and the volatile solids was 0.726 g VS/g dry RS. The average moisture content of pretreated CM was 0.052 g water/g raw chicken manure, the average ash content was 0.340 g ash/g dry CM, and the volatile solids was 0.660 g VS/g dry CM.

Long-term lime-air pretreatment (Appendix B) was performed on 80% RS and 20% CM for the fixed-bed fermentation. The average ash content of the mixture was 0.352 g ash/g dry weight and volatile solids content was 0.648 g VS/g dry weight. Other substrates such as bagasse and paper and poplar wood were obtained from the biomass library in Dr. Holtzapple's laboratory.

2.2 Media and Nutrients

The liquid medium was deoxygenated water containing sodium sulfide and cysteine hydrochloride (Appendix C). These reducing agents were added because a low reducing potential could not be created simply by driving off oxygen. Cysteine hydrochloride is a reducing agent that is nonvolatile, inexpensive, and has a good shelf life. Sodium sulfide is also a reducing agent that is inexpensive and a rapid reductant. Dry nutrients (Appendix D) were used in all fermentations.

2.3 Inoculum

Marine inoculum was used from a previous fermentation of sugarcane bagasse/chicken manure (Thanakoses, 2002). The original inoculum was previously collected from the sediments of three coastal swamps in Galveston, Texas (East Beach, Harborside, and Sportman's Road). The sediment was collected from 0.5-m-deep holes and placed into bottles filled with deoxygenated media.

2.4 Inhibitor

Iodoform (CHI_3) solution containing 20 g CHI_3/L ethanol was used as a methanogen inhibitor in this experiment. Iodoform was added individually to each fermentor. Due to light and air sensitivity, the solution was kept in a tinted bottle and capped immediately after use.

2.5 Fermentors

Two types of fermentors were used in this study, which are described below.

Rotary fermentors

The fermentors (Figure 2-1) were made from Beckman 1-L polypropylene centrifuge bottles (98 × 169 mm), Nalgene brand NNI 3120-1010. The bottles were closed with a size 11 rubber stopper with a hole drilled in the middle. A glass tube was inserted through the hole and capped with a rubber septum for gas sampling and release. The release of gas from the fermentors was necessary to prevent explosions because the fermentors could only withstand a pressure of 2 atm. The rubber septum was replaced when there was a visible hole due to frequent gas venting. Two 0.25-in stainless steel tubes with welded ends were also inserted into holes in the stopper. The tubes were used as stirrers to mix the components inside the fermentors. The fermentors were placed in a Wheaton Modular Cell Production Roller Apparatus (Model III) located in a 40°C incubator and were rotated horizontally at 1 rpm.

To establish the culture, a batch fermentation was maintained for 10 d, then the countercurrent fermentation was started. On every transfer, liquid and solid were transferred (Appendix E). After the system reached a steady state (±5 g/L average total acid concentration), fermentation data were collected to determine acid productivity, carboxylic acid concentration, yield, selectivity, conversion, biotic CO_2 productivity, and CH_4 productivity.

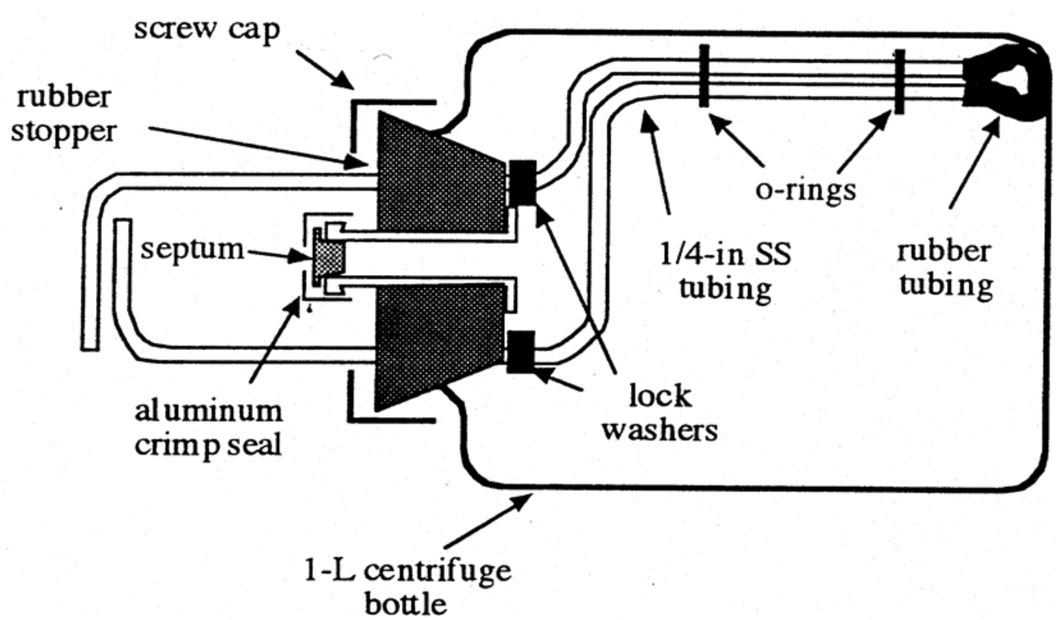


Figure 2-1. Centrifuge bottle bioreactor. (Ross, 1998)

Fixed-bed fermentors

The fixed-bed fermentors were used for both pretreatment and fermentation of biomass (Figure 2-2). The columns (diameter × length = 2 in × 20 in) were made from 2-in and 3-in-diameter PVC pipes and was glued together with 3-in PVC covers with 2-

in holes and 2-in NPT PVC. The outer jacket had heating water inlet controlled to maintain the temperature in the fermentors. The ball valve served two purposes: it introduced air into the fermentor during pretreatment and removed liquid products during fermentation. Two types of covers were used for the fixed-bed fermentors: PVC cover with ¼-inch NPT outlet and rubber stopper. The PVC cover with ¼-inch NPT cover was used for pretreatment and the rubber stopper for fermentation. Air for pretreatment was passed through a cylinder within the tank containing water and lime to scrub CO_2 and saturate the air. Air was purged through the columns; its rate was monitored and estimated by bubbling it through a water-filled tube. The number of bubbles was counted to determine the amount of air going through each column.

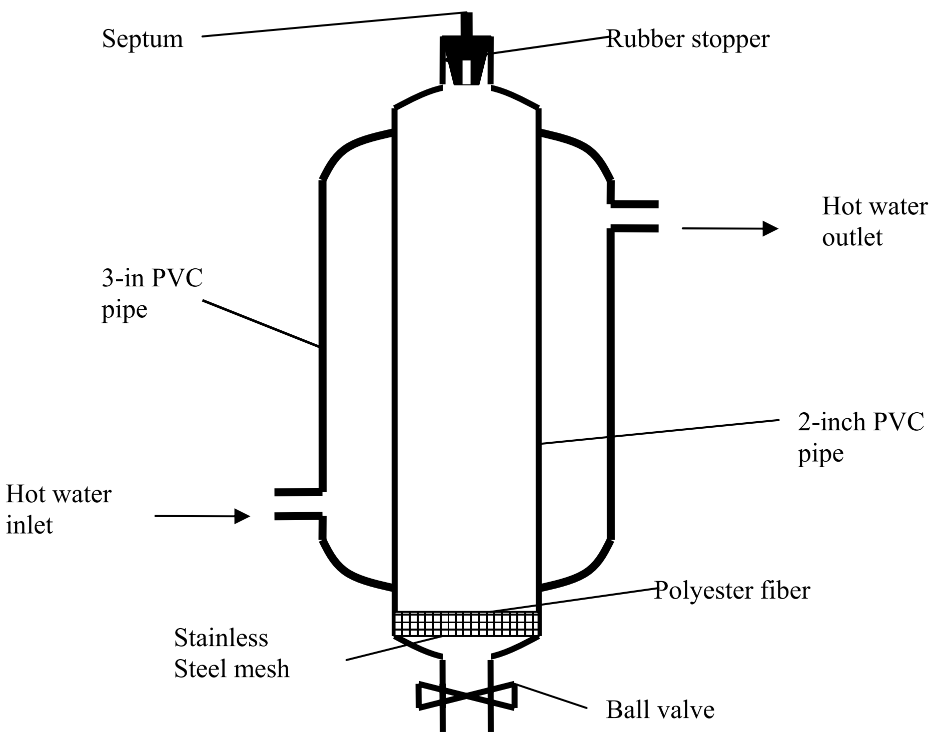


Figure 2-2. Fixed-bed fermentor.

The fermentors had a water heating and circulation system (Figure 2-3). The temperature control system had a temperature controller (1/16 DIN, OMEGA), a thermocouple (KTSS-18G-18, OMEGA), a heating element (1.5 kW, 120 V), a solid-state relay (RSSDN-25A, Idec Co.), fuses (12.5 A and ¼ A), and a main switch. The water circulator consisted of a centrifugal pump (3/4 hp, TEEL, USA), a water tank (8 gallon, Nalgene Co., USA), and a manifold having inputs and return lines.

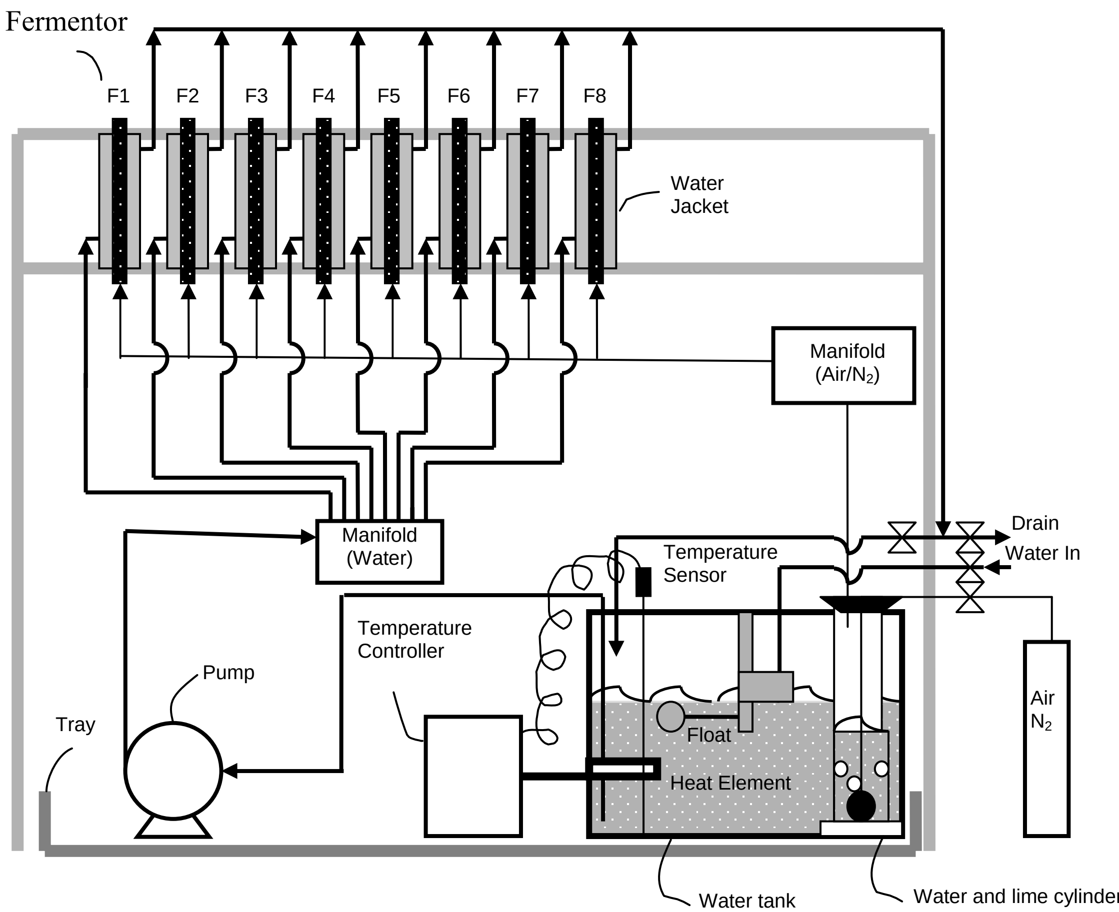


Figure 2-3. Jacketed fermentor system.

2.6 Analytical Methods

The volume of gas produced during fermentation was measured using an inverted graduated glass cylinder apparatus (water displacement apparatus) (Figure 2-4) that was filled with a solution of 30% $CaCl_2$ solution. The $CaCl_2$ minimized microbial growth, reduced water evaporation, and prevented CO_2 adsorption. Gas was released by injecting a needle through the rubber septum on the fermentor. The released gases displaced liquid in the inverted glass cylinder. The inside diameter of the cylinder was 5 cm and this was used with measured height of water displacement to calculate volume of gas displaced by water.

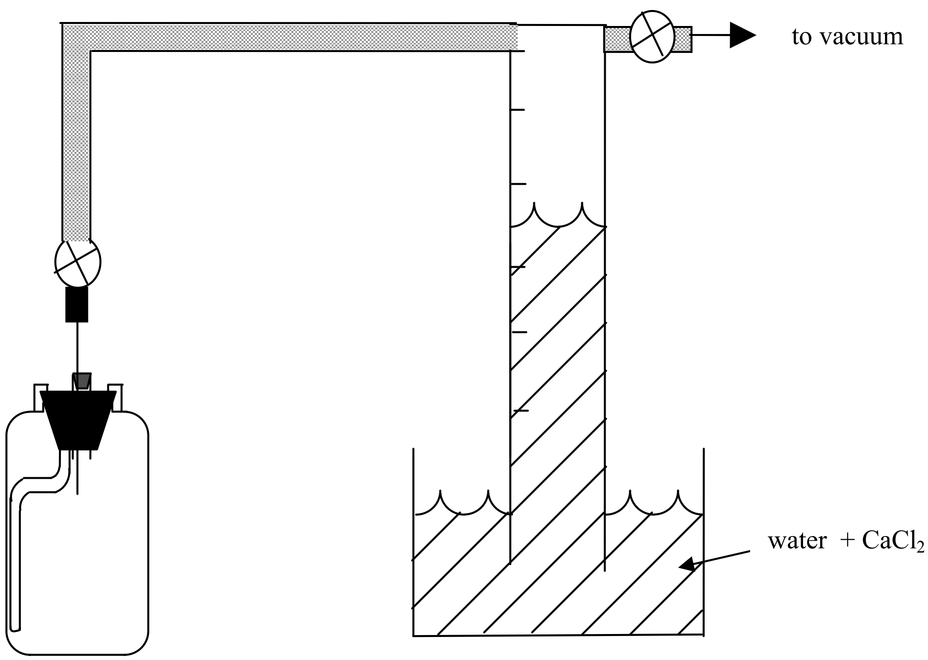


Figure 2-4. Water displacement apparatus.

A gas chromatograph (Agilent 6890 series) with thermal conductivity detector (TCD) was used to determine the methane and carbon dioxide composition of the fermentation gas. Samples were taken directly from the fermentors using a 5-mL syringe. To calibrate the samples, a standard gas mixture of carbon dioxide (29.99 mol%), methane (10.06 mol%), and the balance nitrogen was used.

Carbon dioxide produced during fermentation is the sum of biotic and abiotic carbon dioxide. Abiotic CO_2 is produced by neutralizing the carboxylic acids with calcium carbonate and biotic CO_2 is produced from the fermentation. It is assumed that for every 2 moles of acid produced in the fermentor, 1 mole of abiotic CO_2 is produced. The biotic CO_2 produced directly from the fermentation was calculated by subtracting abiotic CO_2 from total CO_2. Only biotic CO_2 was used in the mass balance calculations.

During each transfer schedule, liquid from Fermentor 1 and solids from Fermentor 4 were collected. A liquid sample (~3 mL) was taken from Fermentor 1 and analyzed for carboxylic acid concentration. The remaining liquid collected from Fermentor 1 was analyzed for volatile solids. The solids collected from Fermentor 4 were analyzed for undigested volatile solids. Acid analysis was performed using an Agilent 6890 gas chromatograph with capillary column (J&W Scientific, model DB-FFAP). It was operated with a flame ionization detector (FID) and an Agilent 7683 Series Injector. The oven temperature in the GC increased from 50°C to 200°C at 20°C/min and was held an additional 1 min at 200°C. Liquid samples were mixed with 1.162 g/L of internal standard (4-methyl-n-valeric acid) and acidified with 3-M phosphoric acid (Appendix F). Volatile solids in all samples were determined by first drying the samples at 105°C and then ashing at 550°C in an oven (Appendix G).

2.7 Mass Balance

Mass balance closure on the entire system was calculated over the steady-state period. The mass balance closure was calculated as

$$\text{Closure} = \frac{\text{Mass out}}{\text{Mass in} + \text{Water of hydrolysis}} \tag{2-1}$$

$$\text{Closure} = \frac{\text{Undigested VS} + \text{Dissolved VS} + \text{Acids} + \text{Biotic } CO_2 + CH_4}{\text{VS in} + \text{Water of hydrolysis}} \tag{2-2}$$

Theoretically, the system should have a 100% closure; in practice, human errors in measurement and transfer processes caused some discrepancies. During cellulose hydrolysis, a mole of water is gained per mole of monomer resulting in mass increase. Ross (1998) suggested that biomass could be represented as cellulose, with a monomer weight of 162 g/mol. The water of hydrolysis was calculated as:

$$\text{Water of hydrolysis} = \text{VS digested} \times \frac{18}{162} \tag{2-3}$$

The following definitions were used:

$$\text{Volatile solids (VS)} = \text{Dry weight} - \text{Ash weight} \tag{2-4}$$

$$\text{Conversion } (x) = \frac{\text{VS digested}}{\text{VS fed}} \tag{2-5}$$

$$\text{Yield } (y) = \frac{\text{Total carboxylic acids produced}}{\text{VS fed}} \tag{2-6}$$

$$\text{Total acid productivity } (p) = \frac{\text{Total carboxylic acids produced}}{\text{Total liquid volume in all fermentors} \cdot \text{time}} \tag{2-7}$$

$$\text{Total acid selectivity} = \frac{\text{Total carboxylic acids produced}}{\text{VS digested}} \tag{2-8}$$

$$\text{Liquid residence time (LRT)} = \frac{\text{Total liquid in all fermentors}}{\text{Flow rate of liquid out of the fermentor train}} \tag{2-9}$$

$$\text{Volatile solids loading rate (VSLR)} = \frac{\text{VS fed to the system}}{\text{Total liquid in all fermentors} \cdot \text{time}} \tag{2-10}$$

2.8 Continuum Particle Distribution Model (CPDM)

The Continuum Particle Distribution Model (CPDM) can be used to quantify the kinetics of a reaction occurring at the interface between solid and fluid phases. Examples of such reactions are i) microbial conversion of lignocellulose to volatile fatty acids, 2) enzymatic hydrolysis of lignocellulose, 3) microbial coal desulfurization, 4) reactive and non-reactive extraction of soil contaminants, and 5) combustion of high-ash coal. This method allows laboratory data to be modeled mechanistically, or empirically to simulate different reactor configurations (Loescher, 1996).

The conventional approach to modeling solid/fluid reactions is the Residence Time Distribution (RTD) model. Although RTD solves the same class of problems as CPDM, it has disadvantages. When the interfacial reaction rate depends on the fluid and solid phases, RTD is difficult to apply because the relationship between reactivity and residence time is not unique for all fluid phase driving forces (Kunii and Levenspiel, 1969). In contrast, CPDM separates fluid and solid dependencies explicitly. In RTD, one must account for particles with residence times between 0 and infinity, which requires that an arbitrary upper bound on time must be assumed. Unlike RTD, CPDM follows particles that are contained in the closed conversion domain from 0 to 1 (Loescher, 1996).

The concept of "continuum particle" is used to avoid the difficulties of tracking the geometry of individual discrete particles. Loescher, 1996 defined the continuum particle as 1 g of solids in the initial unreacted state and Ross, 1998 defined it as 1 g of volatile solids entering the fermentor. The particle concentration (particles/L) S_o is related to the particle distribution function as shown in Equation 2-11.

$$S_o = \int_0^1 \hat{n}(x)dx \qquad (2\text{-}11)$$

The total reaction rate, r is related to the specific rate ($\hat{r}$) as a function of particle conversion and product concentrations A (Equation 2-12). The specific rate $\hat{r}(x, A)$ contains information about the reacting system and products and $\hat{n}(x)$ contains information about substrate concentrations and conversions.

$$r = \int_0^1 \hat{r}(x, A)\hat{n}(x)dx \qquad (2\text{-}12)$$

For a batch reaction, all particles have the same conversion. Therefore $\hat{n}(x)=0$ everywhere except at x'.

$$n_o = \int_0^1 \hat{n}(x)dx = \lim_{\varepsilon \to 0} \int_{x'-\varepsilon}^{x'+\varepsilon} \hat{n}(x)dx \tag{2-13}$$

The Dirac delta function can be used to represent the distribution function as in Equation 2-14.

$$\hat{n}(x) = S_o \delta(x - x') \tag{2-14}$$

Substituting the particle distribution function into the rate equation gives Equation 2-15.

$$r = \int_0^1 \hat{r}(x, A)\hat{n}(x)dx = \int_0^1 \hat{r}(x, A)S_o\delta(x - x')dx = \hat{r}(x', A)S_o \tag{2-15}$$

Equation 2-15 shows that the total reaction rate r is related to the specific reaction rate $\hat{r}(x', A)$ by the initial particle concentration. Therefore, the specific rate $\hat{r}(x', A)$ can be measured by performing batch runs at different initial solid loadings.

Countercurrent fermentation

CPDM was used to simulate data for the countercurrent fermentation using data collected from batch fermentations. Batch experiments at varying initial substrate concentrations (20, 40, 70, 100, and 100^+ g dry substrate/L liquid) were used to obtain the data. The 100 and 100^+ fermentors had the same initial substrate concentrations, but the 100^+ fermentor contained a medium with a mixture of carboxylate salts (70 wt% calcium acetate, 20 wt% calcium propionate, and 10% calcium butyrate) at a concentration of 20 g carboxylic acids/L of liquid. The inoculum for batch fermentations was taken from a steady-state countercurrent fermentation on the same substrates. Deoxygenated water was used for this fermentation and other components, such as urea, dry nutrient, and calcium carbonate, were added initially to the fermentors. To prevent methane production, iodoform was added continuously. Liquid samples were taken daily from the five batches. Reaction rates at varying acid concentrations and biomass digestion were determined from the batch data.

The liquid samples were analyzed for carboxylic acid concentrations using the GC and the results were converted to acetic acid equivalent (Appendix H) (α):

$$\alpha \text{ (mol/L)} = \text{acetic (mol/L)} + 1.75 \times \text{propionic (mol/L)} + 2.5 \times \text{butyric (mol/L)} + 3.25 \times \text{valeric (mol/L)} + 4.0 \times \text{caprioc (mol/L)} + 4.75 \times \text{heptanoic (mol/L)} \quad (2\text{-}16)$$

On a mass basis, acetic acid equivalents can be expressed as:

$$A_e \text{ (g/L)} = 60.05 \text{ (g/mol)} \times \alpha \text{ (mol/L)} \quad (2\text{-}17)$$

Acetic acid equivalents are based on the reducing power of the acids produced from the fermentation and allows the various acid products to be expressed on a common basis (Datta, 1981). The acetic acid equivalents (A_e) from each of the five batch experiment was fit to the equation

$$A_e = a + \frac{bt}{1+ct} \quad (2\text{-}18)$$

where t is the time (d) of fermentation, and a, b, and c are constants fit by least squares analysis. The rate r was obtained by differentiating Equation 2-18.

$$r = \frac{d(A_e)}{dt} = \frac{b}{(1+ct)^2} \quad (2\text{-}19)$$

The specific rate, $\hat{r}$ (g A_e produced/(g VS·d)) was determined from Equation 2-15 by dividing r by the initial amount of substrate concentration, S_o (g VS/L) in each of the five fermentors:

$$\hat{r} = \frac{r}{S_o} \quad (2\text{-}15)$$

The predicted rate, $\hat{r}_{pred}$, was obtained from Equation 2-20, where the rate of acid production depends on volatile solids conversion (x) and product concentration (A_e):

$$\hat{r}_{pred} = \frac{e(1-x)^f}{1+g[\phi A_e]^h} \quad (2\text{-}20)$$

Least squares analysis was used to determine the empirical parameter constants e, f, g, and h for $\hat{r}_{pred}$ (Equation 2-20) from the specific rate $\hat{r}$ (Equation 2-15). A_e was converted back to carboxylic acid concentration by ϕ (the ratio of total grams of actual acids to grams of A_e). The $(1-x)$ term in the numerator of Equation 2-20 is the conversion penalty function by South and Lynd, 1994.

The conversion term $(1-x)$ in the numerator (Equation 2-20) shows that as the biomass is digested, the reaction rate decreases because the less reactive components remain. The acid concentration in the denominator (A_e) shows the inhibitory effect on the microorganisms when product concentration is high.

The conversion in the function, x was calculated using

$$x(t) = \frac{A_e(t) - A_e(t=0)}{S_o \cdot \sigma} \tag{2-21}$$

where,

σ = selectivity (g A_e produced/g VS digested)

S_o = the initial substrate concentration (g VS/L)

The selectivity σ for Equation 2-21 was calculated from the selectivity s (g total acids produced/g VS digested) determined in the countercurrent experiment.

$$s = \phi\sigma \tag{2-22}$$

Equation (2-20) was used in a Mathematica program (Appendix I) to predict acetic acid equivalent concentration (A_e) and conversion (x) for the countercurrent fermentation at various VSLR and LRT. A_e was converted back to carboxylic acid concentration by multiplying by ϕ.

Fixed-bed fermentors

Fixed-bed fermentors are similar to batch reactors because solids are not moved and fresh feed is not added. The only difference is the flow of liquid from one fermentor to the other. The simulation of a batch reactor using CPDM is the reverse of what has been done to obtain the rate equation. From the general mass balance equation,

$$\text{Accumulation} = \text{Input} - \text{Output} + \text{Generation} - \text{Consumption} \qquad (2\text{-}23)$$

For a batch reactor, it is easy to use product accumulation to obtain the rate equation. However, for a fixed-bed system where liquid products are moved from one fermentor to another, the Generation term was used to account for all the products from the initial biomass.

$$\text{Generation} = \text{Accumulation} + \text{Output} - \text{Input} + \text{Consumption}$$

The products from the fermentors were converted to acetic acid equivalents as was done for countercurrent fermentation and the total product generated/time was obtained. Just like the countercurrent fermentation, the data were fit to Equation 2-20 and the conversion was calculated as in Equation 2-21. With the rate equation and initial conditions, it is possible to determine the product concentrations. The acid concentrations in each fermentor were modeled taking the flow in the fermentors into consideration (Appendix J). The predicted acid concentrations were compared with fermentation data.

The 'round robin' fermentation system (Figure 2-5) can be used to obtain a steady total acid concentration. The fresh liquid is added to F1 and products from F1 to F2, F2 to F3, F3 to F4, and F4 to F5. The final product is taken from F5. To maintain a steady total acid concentration, a new fermentor F6 is pretreated and is ready to replace a fully digested fermentor. When the biomass in the first fermentor becomes fully digested, the liquid flow is re-routed, fresh liquid now goes to F2 and products from F2 to F3, F3 to F4, F4 to F5, and F5 to F6 (Figure 2-5). The final product is now taken from F6. The process is repeated anytime a fermentor becomes fully digested.

CPDM used on the fixed bed fermentation was extended to the 'round robin' fermentation system (Appendix K). The liquid flow and re-routing is accounted for in modeling the 'round robin' system.

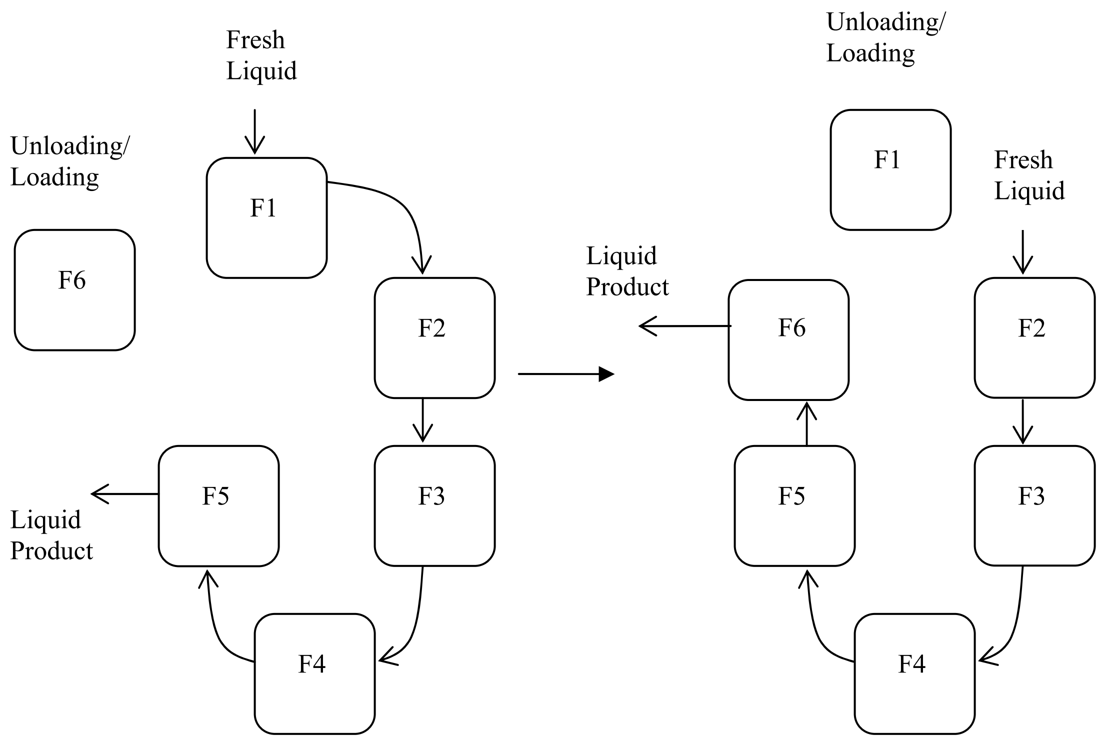


Figure 2-5. ‘Round robin’ fermentation system.

2.9 Statistical Methods

The “solver” in Microsoft Excel was used to obtain the model fit parameters in Equations 2-18 and 2-20. The sum of the mean-square error between experimental and predicted values was minimized to obtain the model parameters.

CHAPTER III
RICE STRAW COUNTERCURRENT FERMENTATION

In this study, experiments were performed using 80% lime-treated rice straw and 20% lime-treated chicken manure as substrates in the rotary fermentors. The carboxylic acid productivity at varying liquid residence time (LRT) and volatile solid loading rate (VSLR) in the countercurrent fermentation were investigated. Moreover, continuum particle distribution model (CPDM) was used to predict the experimental results as well as at other conditions.

3.1 Fermentation Conditions

The fermentations were performed at 40^{o}C. Anaerobic conditions were maintained by purging with nitrogen whenever fermentors were opened. Four fermentors were started as a batch fermentation with 80% pretreated rice straw and 20% pretreated chicken manure, calcium carbonate, urea, dry nutrients, and deoxygenated water. Countercurrent fermentation was initiated after batch culture. Every 2 days, liquid/solid were transferred (Figure 1-7) and 2 g of $CaCO_3$ were added to each fermentor to neutralize the carboxylic acids.

A series of six countercurrent fermentation experiments was performed at various combinations of volatile solid loading rates (VSLR) and liquid residence times (LRT). The operating parameters for the fermentation trains are shown in Table 1. The single-centrifuge procedure, where liquids are transferred in a single step, was used (Aiello-Mazzarri, 2002). After the system reached a steady state (±5 g/L average total acid concentration), fermentation data were collected for at least 10 transfers to determine acid productivity, carboxylic acid concentration, yield, selectivity, conversion, biotic CO_2 productivity, and CH_4 productivity. The total liquid in all fermentors was determined by first centrifuging each fermentor in a train and separating the solid from the liquid. The liquid content of the solid cake from each fermentor was determined as well as the liquid volume from each fermentor. The sum of free liquid after centrifugation in each fermentor as well as liquid remaining in the cake constitute the total liquid in the train.

Train A

Four batch fermentations were initiated by adding 32 g of pretreated rice straw (RS), 8 g of pretreated chicken manure (CM), 3 g of $CaCO_3$, 0.2 g of nutrients, 0.1 g of urea, and 120 μL of iodoform solution (20 g/L of iodoform dissolved in ethanol) (Ross, 1998) to each of four rotary fermentors with 240 mL of anaerobic medium and 60 mL of inoculum. Inocula were taken from a previous batch of bagasse and chicken manure fermentation using marine microorganisms. On each transfer with Train A, 16 g of RS, 4 g of CM, 2 g of $CaCO_3$, 0.2 g of nutrients, and 80 μL of iodoform solution were added to F1. $CaCO_3$ (2 g), 0.2 g of nutrients, and 40 μL of iodoform solution (20 g/L of iodoform dissolved in ethanol) were added to F2, F3, and F4. The transfers of solids and liquid were performed as shown in Figure 1-7. Fresh liquid medium (100 mL) was added to F4 on each transfer and 0.1 g of urea was added as a nitrogen source (if pH < 6.0). The transfers of liquids and solids were done at 2-day intervals for this train. The total acid concentration profile at steady state is shown in Figure 3-1.

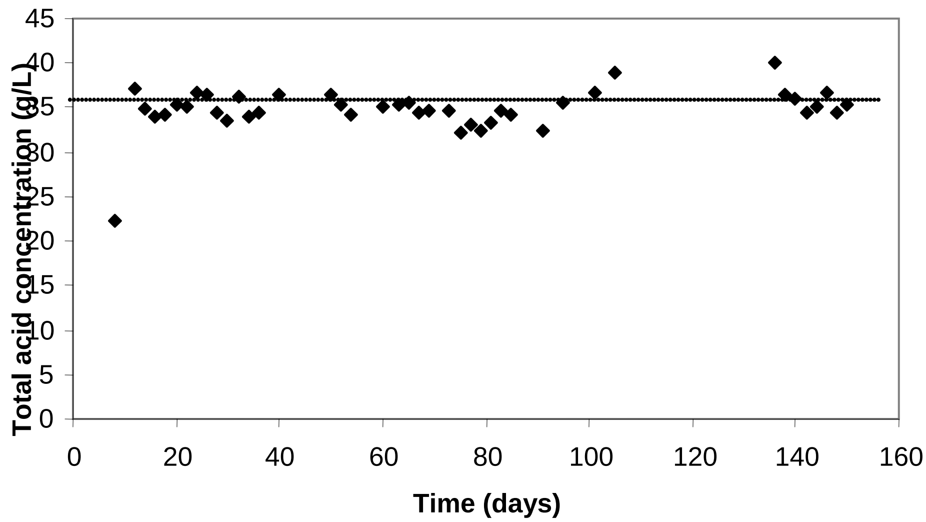


Figure 3-1. Total acid concentration from F1 in Train A.

Train B

Train B was a continuation of Train A but using a different feed rate. On each transfer with Train B, 8 g of RS, 2 g of CM, 2 g of $CaCO_3$, 0.2 g of nutrients, and 80 μL of iodoform solution were added to F1. $CaCO_3$ (2 g), 0.2 g of nutrients, and 40 μL of iodoform solution (20 g/L of iodoform dissolved in ethanol) were added to F2, F3, and F4. The transfers of solids and liquid were performed as in Train A. Fresh liquid medium (100 mL) was added to F4 on each transfer and 0.1 g of urea was added as a nitrogen source (if pH < 6.0). The transfers of liquids and solids were done at 2-day intervals for this train. The total acid concentration profile at steady state is shown in Figure 3-2.

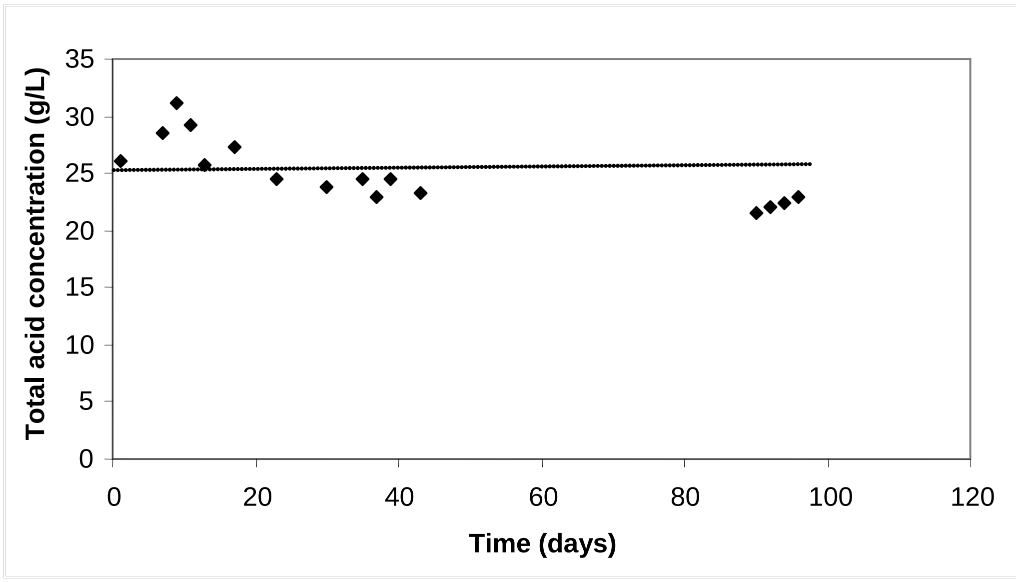


Figure 3-2. Total acid concentration from F1 in Train B.

Train C

On each transfer with Train C, 16 g of RS, 4 g of CM, 2 g of $CaCO_3$, 0.2 g of nutrients, and 80 μL of iodoform solution were added to F1. $CaCO_3$ (2 g), 0.2 g of nutrients, and 40 μL of iodoform solution (20 g/L of iodoform dissolved in ethanol) were added to F2, F3, and F4. The transfers of solids and liquid were performed as in Train A. Fresh liquid medium (100 mL) was added to F4 on each transfer and 0.1 g of urea was added as a nitrogen source (if pH < 6.0). The transfers of liquids and solids were done at 2-day intervals for this train. The total acid concentration profile at steady state is shown in Figure 3-3.

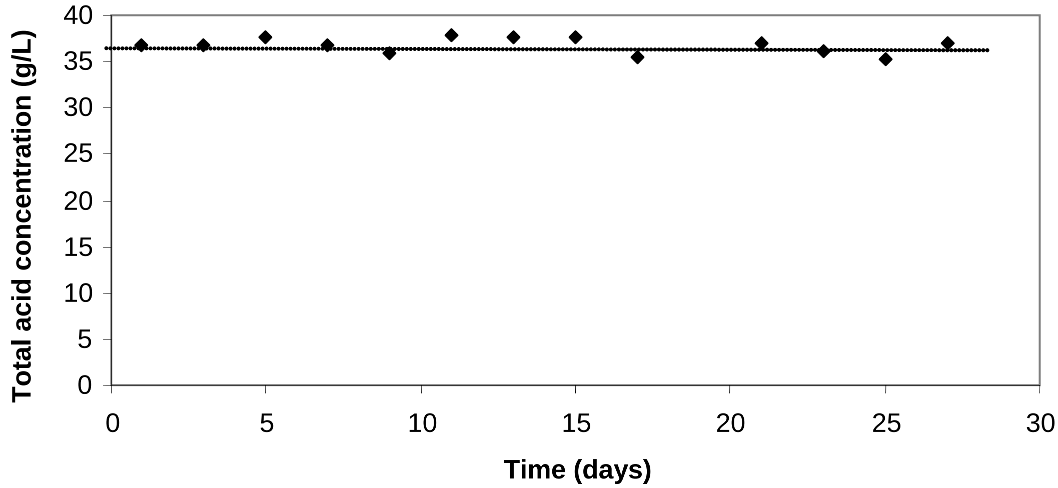


Figure 3-3. Total acid concentration from F1 in Train C.

Train D

On each transfer with Train D, 24 g of RS, 8 g of CM, 2 g of $CaCO_3$, 0.2 g of nutrients, and 80 μL of iodoform solution were added to F1. $CaCO_3$ (2 g), 0.2 g of nutrients, and 40 μL of iodoform solution (20 g/L of iodoform dissolved in ethanol) were added to F2, F3, and F4. The transfers of solids and liquid were performed as in Train A. Fresh liquid medium (100 mL) was added to F4 on each transfer and 0.1 g of urea was added as a nitrogen source (if pH $<$ 6.0). The transfers of liquids and solids were done at 2-day intervals for this train. The total acid concentration profile at steady state is shown in Figure 3-4.

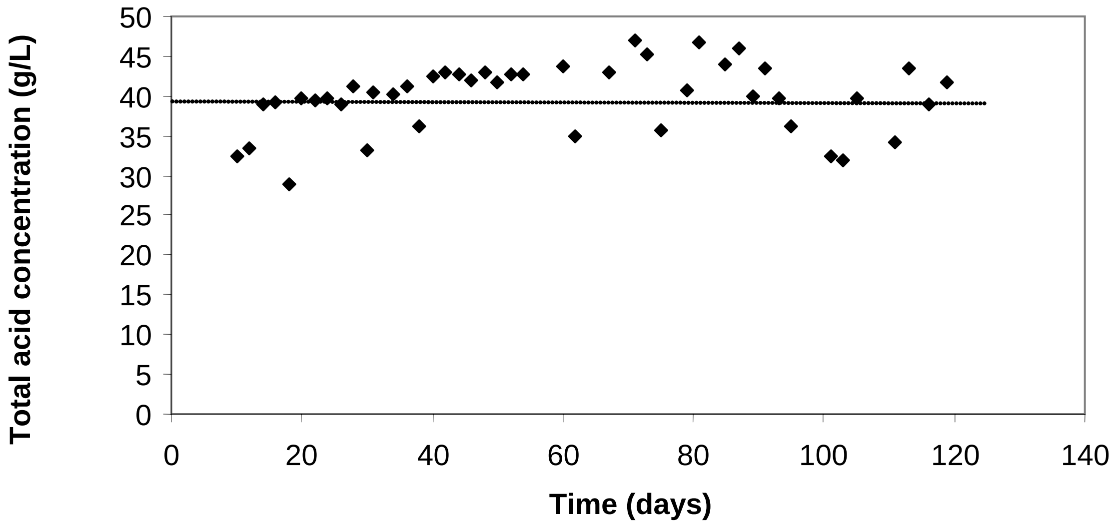


Figure 3-4. Total acid concentration from F1 in Train D.

Train E

On each transfer with Train E, 12 g of RS, 4 g of CM, 2 g of $CaCO_3$, 0.2 g of nutrients, and 80 μL of iodoform solution were added to F1. $CaCO_3$ (2 g), 0.2 g of nutrients, and 40 μL of iodoform solution (20 g/L of iodoform dissolved in ethanol) were added to F2, F3, and F4. The transfers of solids and liquid were performed as in Train A. Fresh liquid medium (100 mL) was added to F4 on each transfer and 0.1 g of urea was added as a nitrogen source (if pH < 6.0). The transfers of liquids and solids were done at 2-day intervals for this train. The total acid concentration profile at steady state is shown in Figure 3-5.

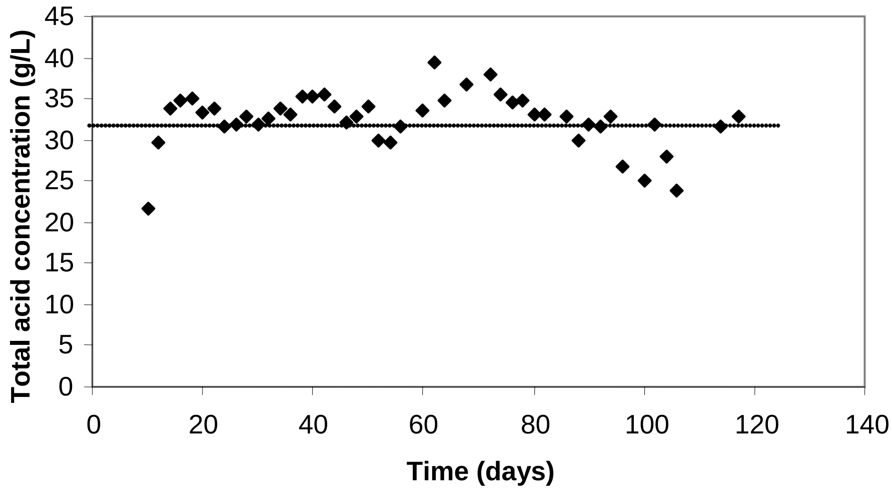


Figure 3-5. Total acid concentration from F1 in Train E.

Train F

On each transfer with Train F, 20.8 g of RS, 5.2 g of CM, 2 g of $CaCO_3$, 0.2 g of nutrients, and 80 μL of iodoform solution were added to F1. $CaCO_3$ (2 g), 0.2 g of nutrients, and 40 μL of iodoform solution (20 g/L of iodoform dissolved in ethanol) were added to F2, F3, and F4. The transfers of solids and liquid were performed as in Train A. Fresh liquid medium (100 mL) was added to F4 on each transfer and 0.1 g of urea was added as a nitrogen source (if pH < 6.0). The transfers of liquids and solids were done at 2-day intervals for this train. The total acid concentration profile at steady state is shown in Figure 3-6.

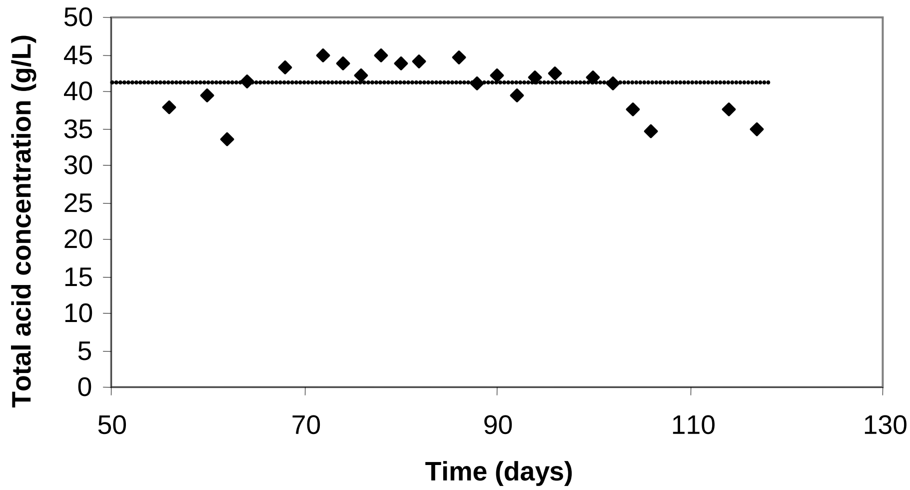


Figure 3-6. Total acid concentration from F1 in Train F.

A summary of the operating conditions for Trains A–F is shown in Table 3-1. The results for the countercurrent fermentation are shown in Table 3-2. The detailed mass balances and acid production from Trains A–F are shown in Appendix N (Tables N3A–N3L). The highest acid productivity of 1.69 g/(L·d) occurred at a concentration of 32.4 g/L in Fermentation Train E (LRT = 19.2 d and VSLR = 5.87 g/(L·d)). This fermentation train had the shortest LRT; Fermentation Train F (LRT = 26.3 d and VSLR = 8.06 g/(L·d)) had the longest LRT. Fermentation Train B (LRT = 23.7 d and VSLR = 3.45 g/(L·d)) with a concentration of 25 g/L had the highest conversion (0.692 g VS digested/g VS fed) and yield (0.29 g total acids/g VS fed). Fermentation Train B had the highest conversion and yield because it had the lowest VSLR, which made more complete use of the biomass. The highest selectivity of 0.57 g total acids/g VS digested was in Fermentation Train C (LRT = 24.0 d and VSLR = 6.47 g/(L·d)).

Table 3-1. Operating parameters for rice straw/chicken manure countercurrent fermentation with marine inoculum

Fermentation Trains	A	B	C	D	E	F
VSLR (g VS/(L of liquid in all fermentors·d))	6.32	3.45	6.47	9.99	5.87	8.06
LRT (days)	23.4	23.7	24.0	24.1	19.2	26.3
VS fed at each transfer (g VS)	13.9	6.96	13.9	22.2	11.1	18.1
Liquid fed to F4 at each transfer (L)	0.1	0.1	0.1	0.1	0.1	0.1
Temperature (^{o}C)	40	40	40	40	40	40
Frequency of transfer	2 days	2 days	2 days	2 days	2 days	2 days
Liquid volume in all fermentors (L)	1.10	1.01	1.08	1.11	0.944	1.12
Iodoform addition rate (mg iodoform added to each fermentor/L of liquid fed to F4)	24	24	24	24	24	24
Urea addition (g urea added to each fermentor/L of liquid fed to F4) (if pH < 6.0)	1.00	1.00	1.00	1.00	1.00	1.00

Table 3-2. Results for rice straw/chicken manure countercurrent fermentation with marine inoculum

Fermentation Trains	A	B	C	D	E	F
Average pH in all fermentors	5.96±0.18	6.36±0.37	5.74±0.06	5.74±0.18	5.80±0.16	5.83±0.17
Total acid productivity (g/(L of liquid in all fermentors·d))	1.47	1.00	1.53	1.63	1.69	1.57
Total acid concentration (g/L)	35.1±1.61	25.0±2.80	36.7±0.850	39.8±4.30	32.4±3.35	40.8±3.37
Acetic acid (wt%)	42.8±2.21	36.1±3.18	48.6±1.28	48.4±4.36	44.0±4.72	50.9±2.56
Propionic acid (wt%)	8.55±2.85	21.3±2.78	3.64±0.18	7.32±4.10	5.46±2.15	8.13±2.80
Butyric acid (wt%)	24.8±1.55	26.7±3.46	27.2±0.49	25.4±2.55	29.9±3.54	23.9±3.41
Valeric acid (wt%)	8.44±1.37	8.84±0.990	4.45±0.445	5.17±1.55	5.59±1.10	4.79±0.80
Caproic acid (wt%)	13.4±2.65	6.11±0.813	15.6±0.720	13.2±2.48	14.5±1.76	11.9±1.65
Heptanoic acid (wt%)	2.01±0.327	0.954±0.433	0.56±0.039	0.47±0.142	0.55±0.112	0.41±0.047
VS digested (g VS/d)	3.54	2.41	2.91	4.13	3.39	3.77
Yield (g total acid/g VS fed)	0.233	0.290	0.237	0.163	0.286	0.194
Selectivity (g total acid/g VS digested)	0.458	0.419	0.568	0.438	0.470	0.466
Conversion (g VS digested/g VS fed)	0.509	0.692	0.417	0.375	0.610	0.417
Biotic CO_2 productivity (g CO_2/(L of liquid in all fermentors·d))	0.433	0.609	0.342	0.166	0.218	0.249
CH_4 productivity (g CH_4/(L of liquid in all fermentors·d))	0.003	0.043	0.015	0.001	0.001	0.0008
Mass balance closure (g VS out/g VS in)	1.19	0.93	1.08	0.961	1.15	1.16

Note: All errors are ± 1 standard deviation

The correlations between VSLR and productivity (p), selectivity (s), conversion (x), and yield (y) are shown in Figures 3-7 to 3-10. The data for the six fermentation trains were fit with a linear regression and the following correlations were obtained:

$$p = 0.0827 \text{ VSLR} + 0.9284 \quad (3\text{-}1)$$

$$s = 0.0015 \text{ VSLR} + 0.4596 \quad (3\text{-}2)$$

$$y = -0.0213 \text{ VSLR} + 0.3763 \quad (3\text{-}3)$$

$$x = -0.0507 \text{ VSLR} + 0.8429 \quad (3\text{-}4)$$

From Figure 3-7, the acid productivity increases as VSLR increases. Figures 3-8 and 3-10 show a decrease in conversion (x), and yield (y) as VSLR increases. Figure 3-9 show that selectivity (s) is essentially constant. At a high VSLR, the microorganisms digest more biomass leading to a high acid productivity; however, yield, and conversion are lower because only a small fraction of the total biomass fed is digested. On the other hand, at low VSLR, the microorganisms are limited by the amount of digestible biomass available. Therefore, the acid productivity is low but the yield, and conversion are higher because the microorganisms consume both the reactive and recalcitrant biomass components.

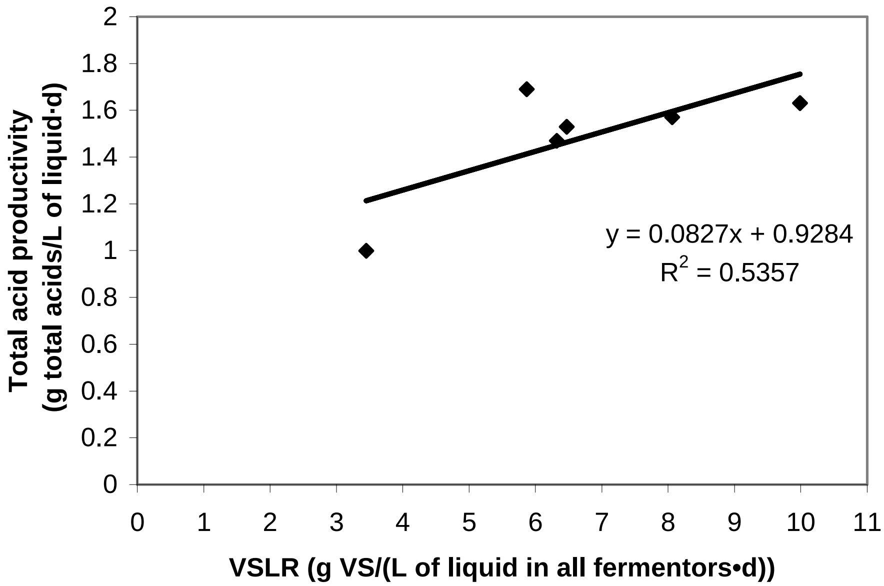


Figure 3-7. Correlation of total acid productivity with volatile solid loading rate.

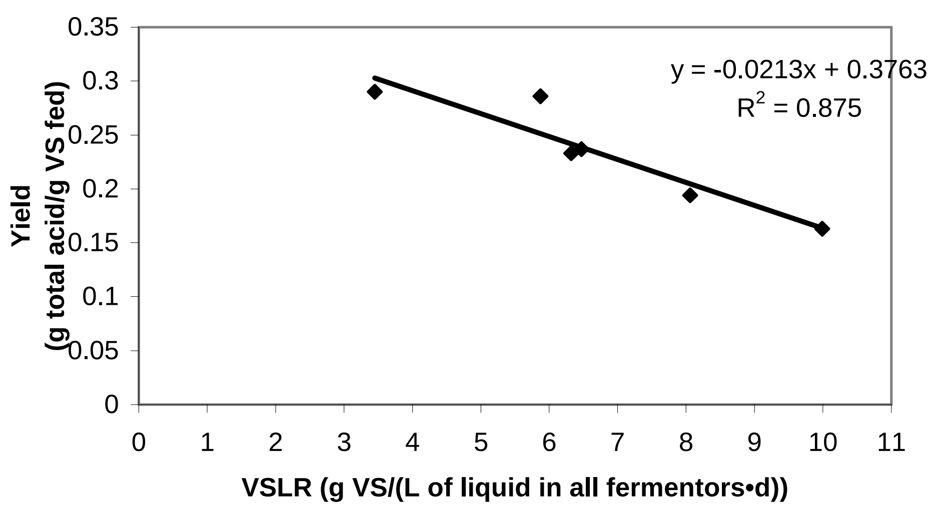


Figure 3-8. Correlation of yield with volatile solid loading rate.

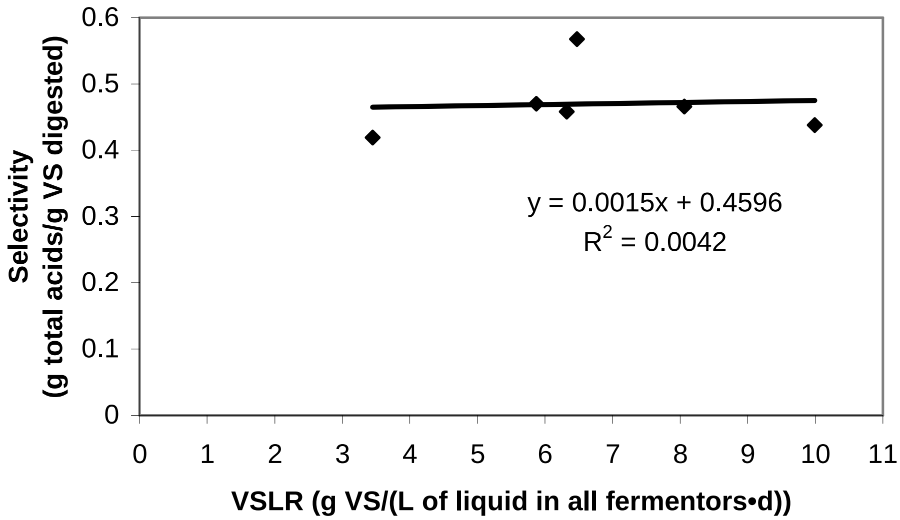


Figure 3-9. Correlation of selectivity with volatile solid loading rate.

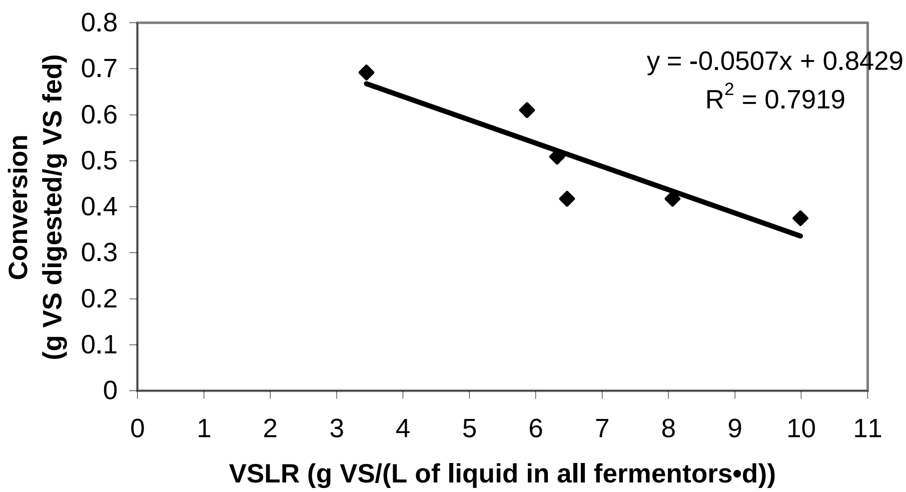


Figure 3-10. Correlation of conversion with volatile solid loading rate.

The mass balance closures for the six fermentation trains are shown in Figures 3-11 to 3-16. The mass balance closure is the ratio of mass exiting the system to mass entering the system. The system should theoretically have 100% closure; however, due to measurement and transfer errors, discrepancies exist in the measurements.

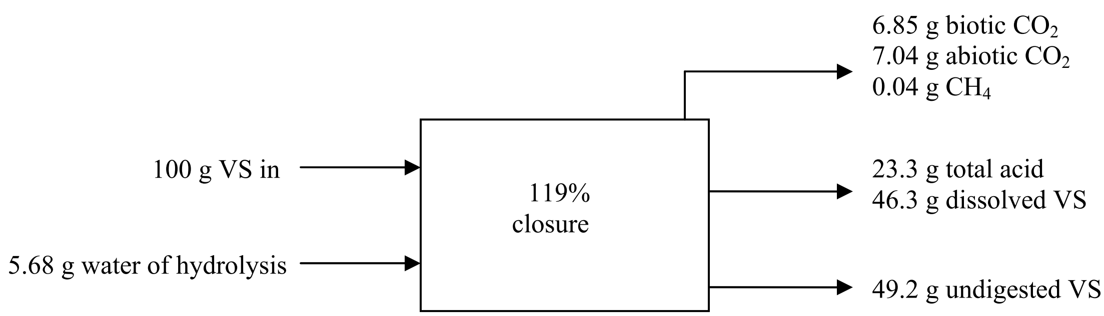


Figure 3-11. Mass balance in Train A.

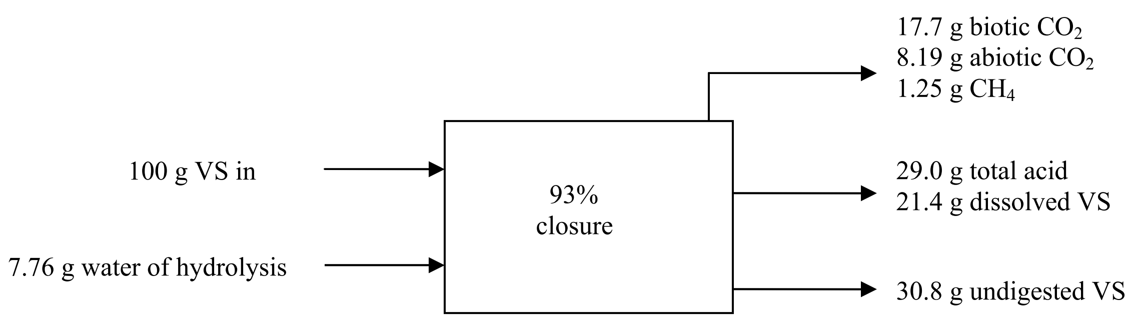


Figure 3-12. Mass balance in Train B.

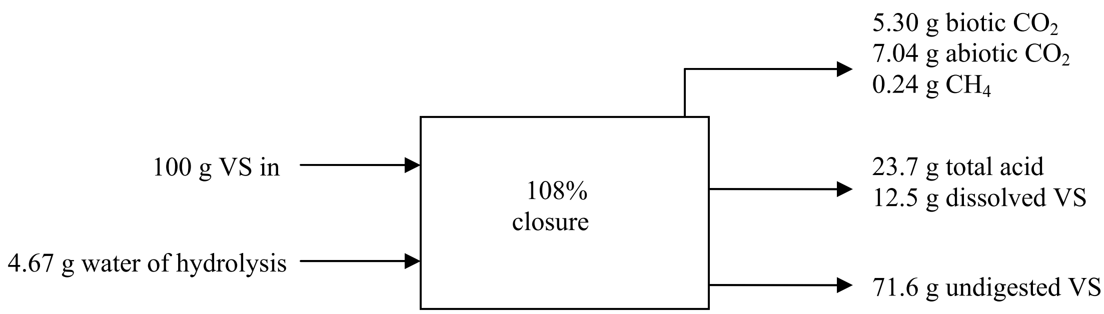


Figure 3-13. Mass balance in Train C.

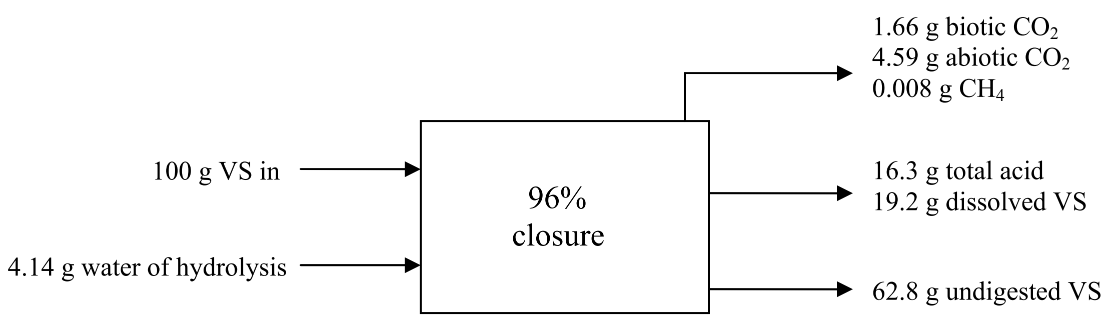


Figure 3-14. Mass balance in Train D.

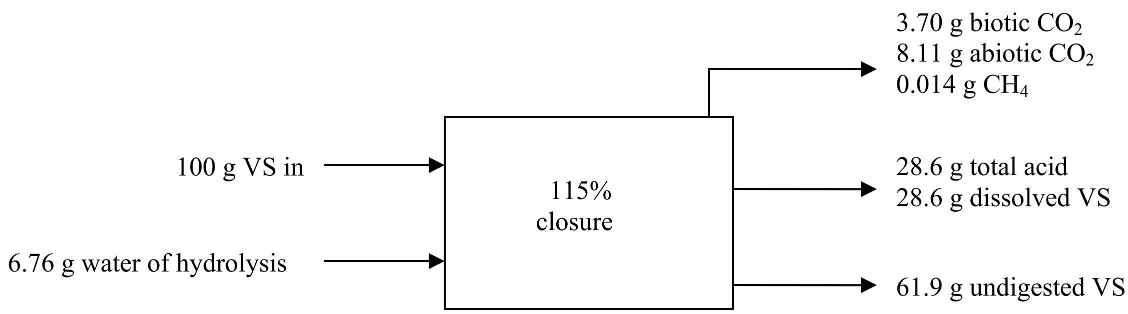


Figure 3-15. Mass balance in Train E.

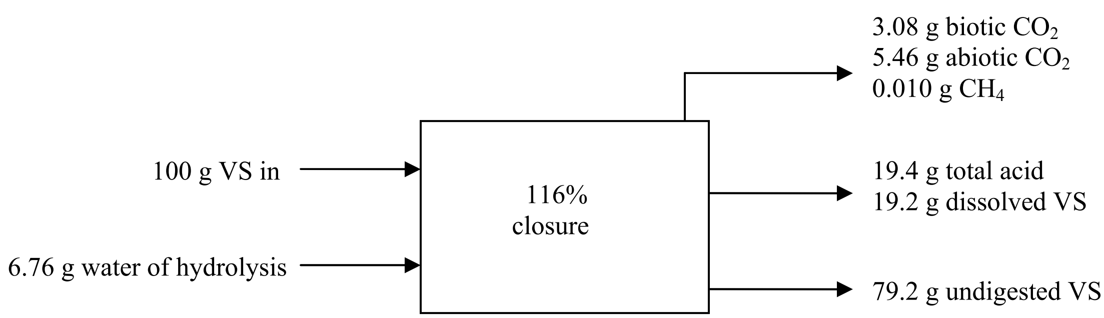


Figure 3-16. Mass balance in Train F.

3.2 Model Development

CPDM was used to predict countercurrent fermentation using data collected from batch fermentations. Batch experiments at varying initial substrate concentrations (20, 40, 70, 100, and 100^+ g dry substrate/L liquid) were used to obtain the data. The 100 and 100^+ fermentors had the same initial substrate concentrations, but the 100^+ fermentor contained a medium with a mixture of carboxylate salts (70 wt% calcium acetate, 20 wt% calcium propionate, and 10 wt% calcium butyrate) at a concentration of 20 g carboxylic acids/L of liquid. The inoculum for batch fermentations was taken from a steady-state countercurrent fermentation on the same substrates. The experimental data are shown in Appendix N (Tables N3M–N3Q).

Deoxygenated water was used for this fermentation and other components, such as urea, dry nutrient, and calcium carbonate, were added initially to the fermentors. To prevent methane production, iodoform was added daily. Liquid samples were taken daily from the five batches. Reaction rates at varying acid concentrations and biomass digestion were determined from the batch data. The graphs for the batch fermentations at varying initial substrate concentrations are shown in Figures 3-17 to 3-21.

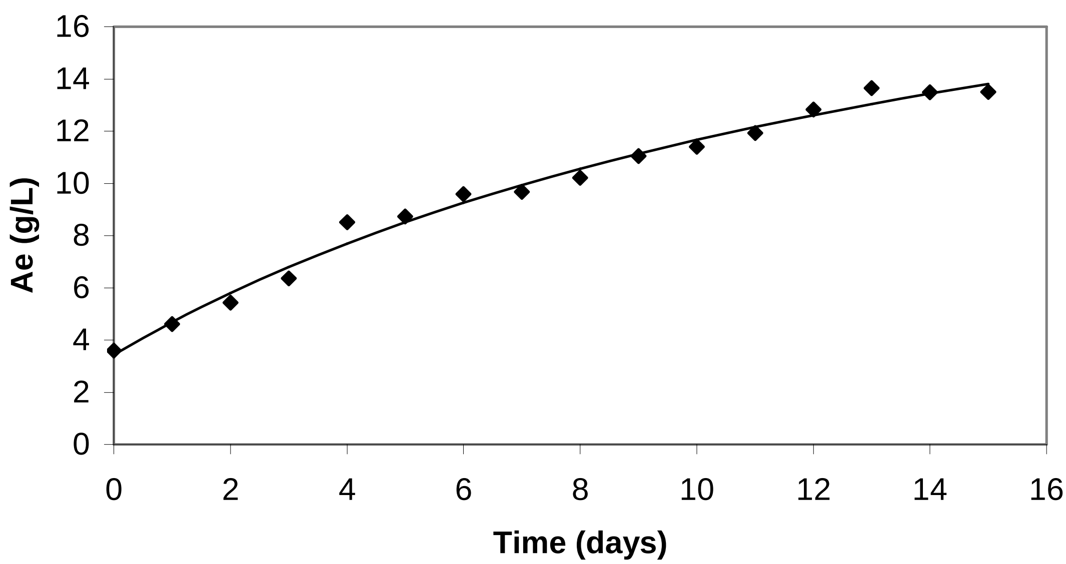


Figure 3-17. Acetic acid equivalent for rice straw/chicken manure batch fermentor (20 g dry substrate/L liquid).

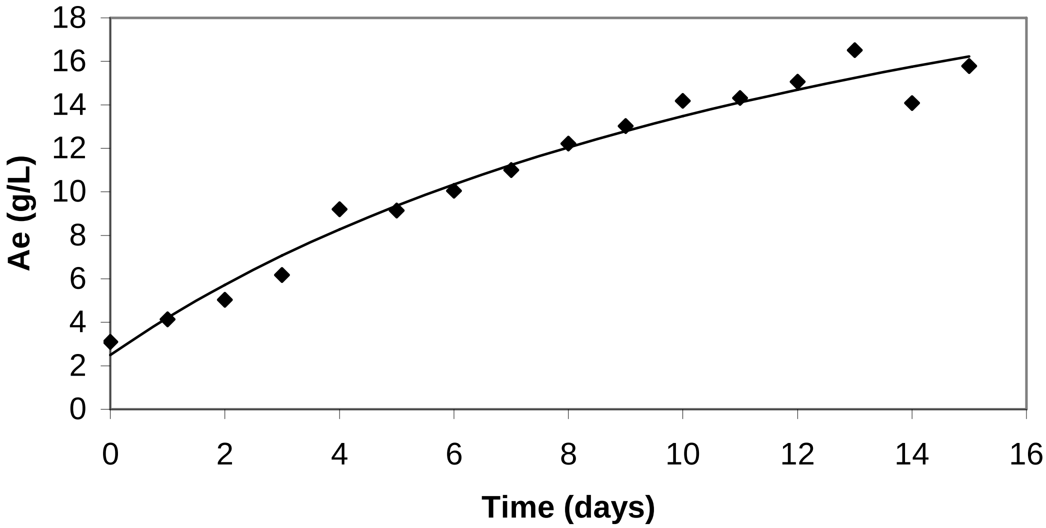


Figure 3-18. Acetic acid equivalent for rice straw/chicken manure batch fermentor (40 g dry substrate/L liquid).

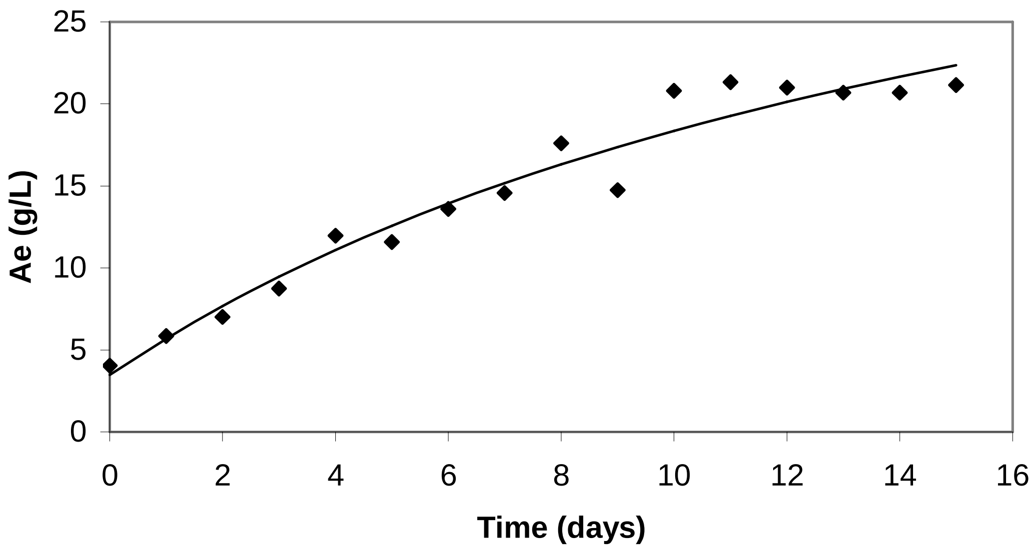


Figure 3-19. Acetic acid equivalent for rice straw/chicken manure batch fermentor (70 g dry substrate/L liquid).

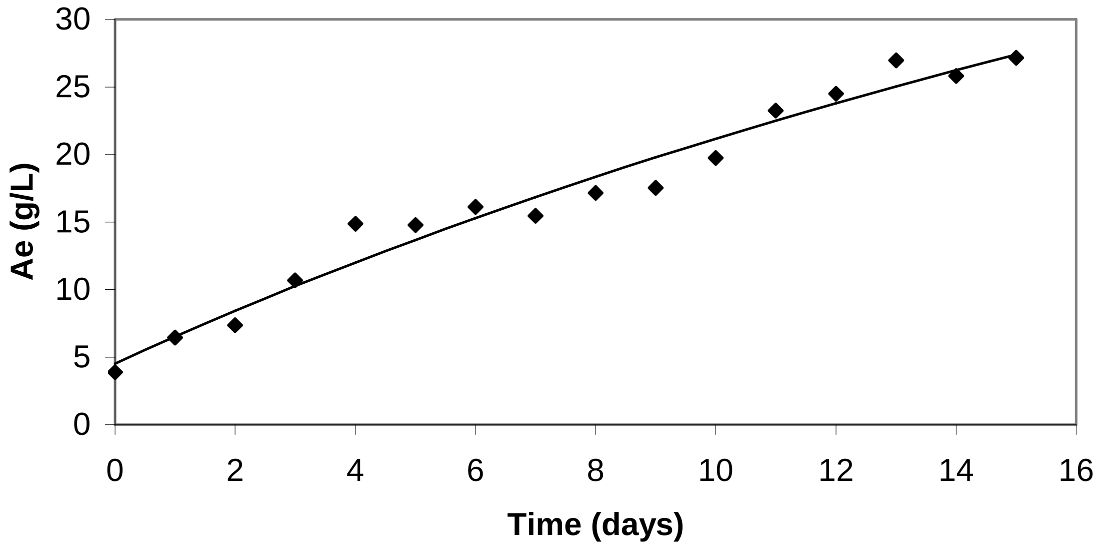


Figure 3-20. Acetic acid equivalent for rice straw/chicken manure batch fermentor (100 g dry substrate/L liquid).

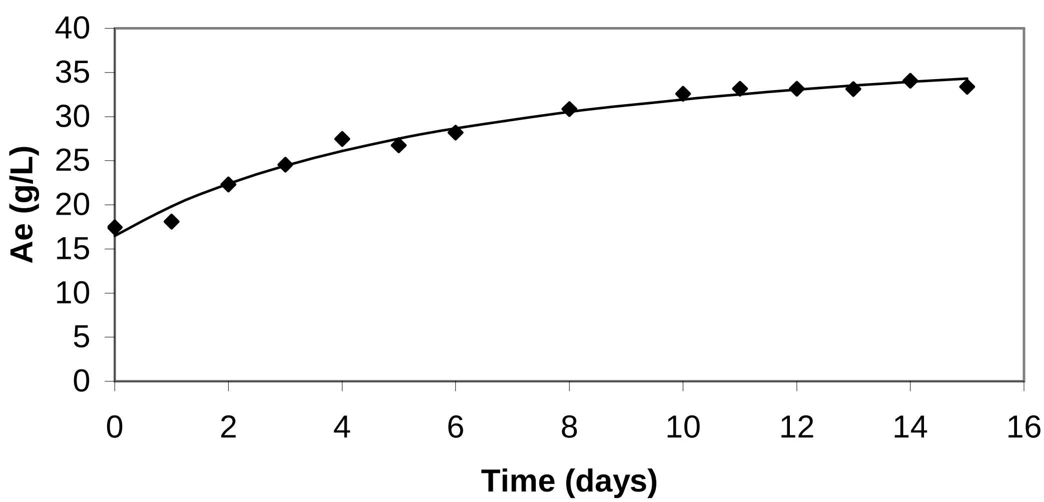


Figure 3-21. Acetic acid equivalent for rice straw/chicken manure batch fermentor (100^+ g dry substrate/L liquid).

The values for a, b, and c at the different initial substrate concentrations are shown in Table 3-3.

Table 3-3. The values of a, b, c in CPDM for rice straw/chicken manure fermentation

Initial substrate concentration (g/L)	a (g/L liquid)	b (g/(L liquid·d))	c (d^{-1})
20	3.44	1.33	0.061
40	2.49	1.83	0.067
70	3.47	2.34	0.057
100	4.51	2.04	0.023
100^{+}	16.48	3.83	0.148

The values of e, f, g, h and other parameter constants used in the Mathematica program (see Appendix I) are shown in Table 3-4. The rate equation obtained for 80% rice straw/20% chicken manure fermentation with marine inoculum is

$$\hat{r}_{pred} = \frac{1.06(1-x)^{3.18}}{1+3.00[\phi A_e]^{0.917}} \tag{3-6}$$

where,

$\hat{r}_{pred}$ = g acetic acid equivalents produced/(g VS·d)

x = dimensionless

ϕ = ratio of g total acid to g acetic acid equivalents

A_e = g acetic acid equivalent produced/L

Table 3-4. Parameter values in CPDM for rice straw/chicken manure fermentation

Parameter	Values
Hold up (g liquid/g VS wet cake)	4.40
Moisture (g liquid/g wet solid)	0.051
Selectivity (g Ae/g VS digested)	0.497
ϕ (g total acid/g Ae)	0.704
Liquid volume (L)	0.269
e (g Ae/g VS·d)	1.06
f (dimensionless)	3.18
g (L/g total acid)$^{1/h}$	3.00
h (dimensionless)	0.917

Table 3-5 compares the experimental total carboxylic acid concentration and conversion to the CPDM predictions. The average error between the experimental and predicted total acid concentrations is 6.41%, and the average error between the experimental and predicted conversion is 6.55%. The highest error in total acid total carboxylic acids is 16.2% and the highest error in conversion is 22.1%. The CPDM model assumes that the selectivity (σ) is constant; however, in actuality the selectivity varies with VSLR (see Equation 2-20). This inherent assumption in CPDM causes errors between the experimental and predicted values.

Table 3-5. Comparison of experimental and predicted carboxylic acid concentration and substrate conversion for rice straw/chicken manure fermentation

Fermentation Trains	A	B	C	D	E	F	Average (%)
Experimental carboxylic acid concentration (g/L)	35.1±1.6	25±2.8	36.7±0.85	39.8±4.3	32.4±3.4	40.8±3.4	
Predicted carboxylic acid concentration (g/L) (CPDM)	35.1	29.06	35.7	36.9	30.6	38.26	
Error** (%)	0	16.24	–2.72	–7.29	–5.55	–6.64	6.41*
Experimental conversion	0.509	0.692	0.417	0.375	0.610	0.417	
Predicted conversion (CPDM)	0.521	0.730	0.509	0.380	0.577	0.428	
Error** (%)	2.36	5.49	22.06	1.33	–5.41	2.64	6.55*

Note: All errors are ± 1 standard deviation
*Average errors are based on absolute value
**Error = (Predicted–Experimental) × 100/ Experimental.

Figure 3-22 shows the CPDM "map" at the average solid concentrations used in this study (129.5 g VS/L of liquid). The "map" predicts a total acid concentration of 40 g/L at LRT of 30 d, VSLR of 8 g/(L·d) and a conversion of 42%. At a VSLR of 2.5 g/(L·d) and LRT of 30 d, a total acid concentration of 30 g/L could be obtained at 80% conversion. Figure 3-23 shows the CPDM "map" for the 80% rice straw/20% chicken manure fermentation system in an industrial fermentor (300 g VS/L of liquid). The CPDM "map" shows that more than 90% conversion could be obtained at a LRT of 10 d and VSLR of 4 g/(L·d). The "map" predicts a total acid concentration of 60 g/L at LRT of 30 d, VSLR of 10 g/(L·d) and a conversion of 50%. At a VSLR of 4 g/(L·d) and LRT of 30 d, a total acid concentration of 50 g/L could be obtained at 80% conversion.

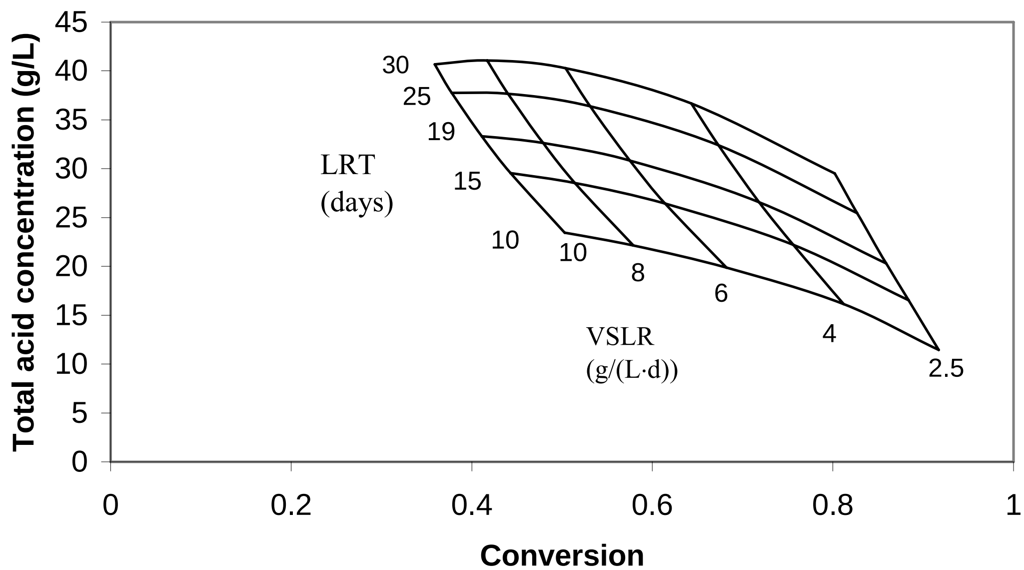


Figure 3-22. The CPDM "map" for rice straw/chicken manure countercurrent fermentation (129.5 g VS/L of liquid).

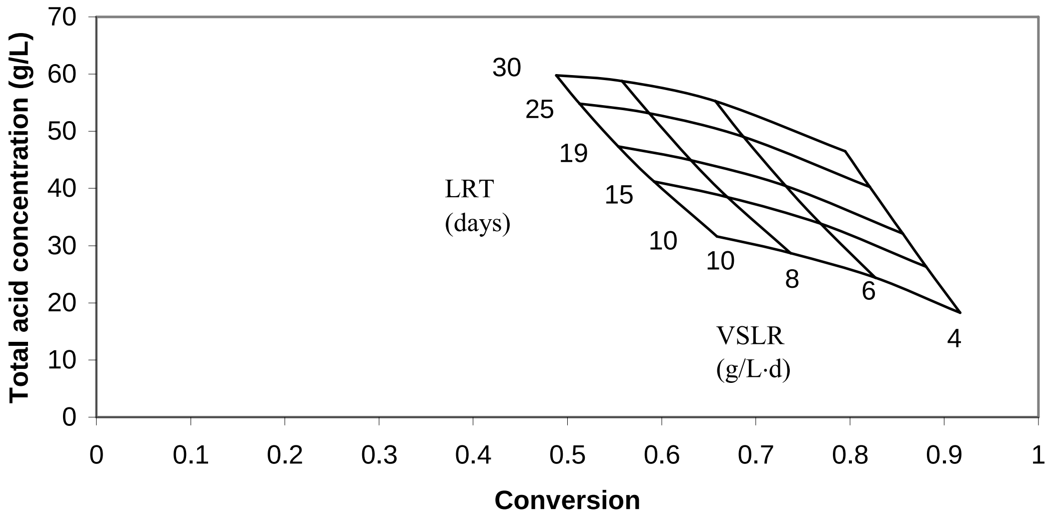


Figure 3-23. The CPDM "map" for rice straw/chicken manure countercurrent fermentation (300 g VS/L of liquid).

3.3 Conclusion

The 80% rice straw/20% chicken manure fermentation had the highest acid productivity of 1.69 g/(L·d) at a total acid concentration of 32.4 g/L. The highest conversion (0.692 g VS digested/g VS fed) and yield (0.29 g total acids/g VS fed) occured at a total acid concentration of 25 g/L. The CPDM model predicted the experimental total acid concentration and conversion at an average error of 6.41% and 6.55% respectively. The CPDM "map" at 300 g VS/L of liquid predicts a total acid concentration of 60 g/L at LRT of 30 d, VSLR of 10 g/(L·d) and a conversion of 50%. At a VSLR of 4 g/(L·d) and LRT of 30 d, a total acid concentration of 50 g/L could be obtained at 80% conversion.

CHAPTER IV
FIXED-BED FERMENTATION

In this section, fixed-bed fermentation was studied using rice straw and chicken manure. The purposes of this study follow:

- To demonstrate that air-lime pretreatment and fermentation can be performed in the same unit.
- To show that good fermentation yields can be obtained even if only liquids are moved from one fermentor to the other.
- The CPDM model can be extended to the fixed-bed fermentation system and the round robin system.

Biomass (rice straw and chicken manure) was treated with lime [$Ca(OH)_2$] within the fixed-bed fermentor at 50^{o}C. The procedure is described in Appendix B. Air was purged through the fixed-bed fermentor; the rate was monitored by bubbling it through a water-filled tube. The number of bubbles was counted to determine the amount of air going through each column. The average airflow through each fixed-bed fermentor was 10.8 cm^3/s. During the pretreatment, the fermentors were checked regularly to ensure that the entire biomass was covered with water. The pH was also monitored continuously. The quantities of materials used in the pretreatment are shown in Table 4-1. Two sets of fermentors were set up with each set consisting of eight fixed-bed fermentors (F1–F8) to constitute two sets of liquid transfer.

After pretreatment for 6 weeks, the air was replaced with nitrogen to create the anaerobic conditions required for fermentation. Biomass samples were taken to determine the moisture content and the volatile solids. Nitrogen was flushed through the system for 3 h before the fermentors were inoculated.

Table 4-1. Pretreatment conditions

Substrate	Quantity
Rice straw (g)	64
Chicken manure (g)	16
Lime (g $Ca(OH)_2$)	16
H_2O (mL)	850
Temp (^oC)	50
Time (wk)	6

4.1 Fermentation Trains A and B

The total volatile solid in Trains A and B before fermentation was 44 g and the total liquid volume was 384 mL. The solids and liquids were evenly distributed among the eight fermentors in the trains. The fermentations were performed under anaerobic conditions at 40^{o}C. Anaerobic medium (100 mL), 0.4 g of dry nutrients, 0.2 g urea, 30 mL of inocula, and 200 μL of iodoform were added. $CaCO_3$ was not added because the average pH was 9.41 prior to fermentation. To maintain anaerobic conditions, nitrogen from a high-pressure liquid nitrogen cylinder was flushed whenever the fermentors were open to the atmosphere. Batch fermentation was performed by adding 0.2 g of dry nutrients, and 160 μL of iodoform solution every 4 days and 0.1 g urea (if pH was less than 6). $CaCO_3$ (2 g) was added every 4 days when the pH was below 7. The gas samples were analyzed to determine the methane content. More iodoform solution (120 μL) was added to fermentors showing high methane content.

After one month, liquid was transferred between the columns followed by the addition of 0.2 g of dry nutrients, 160 μL of iodoform solution every 4 days, and 0.1 g urea (if pH was less than 6). In each set, a fixed volume of liquid was removed from F1 and added to F2, from F2 to F3, and from F3 to F4. Fresh liquid medium was used to replace the liquid from F1 and product was harvested from F4. Liquid volumes of 10 mL and 15 mL were transferred every 4 days for Trains A and B, respectively. This liquid addition rate is estimated to be the amount required to digest all the biomass in all fermentors in approximately 1 year.

The results are shown in Figures 4-1 and 4-2. The detailed experimental results are shown in Appendix N (Tables N4A–N4H). The maximum total acid concentration for F1 in Train A was 34.2 g/L and the maximum acid concentration in F2–F4 was ~44 g/L. The maximum total acid concentration in F1 in Train B was 30.5 g/L and the maximum acid concentration in F2–F4 was ~48 g/L. Although there was almost no difference in the total acid concentrations of F2–F4 in both sets, the times at which the maximum peak occurred increased from F2 to F4 (Figures 4-1 and 4-2, Tables 4-2 and 4-3). Conversions in each of the reactors in Train A varied from 0.821–0.879 g VS digested/g VS fed and the yields were in the range 0.489–0.609 g total acids/g VS fed. Conversions and yields in Train B were 0.741–0.914 g VS digested/g VS fed and 0.563–0.669 g total acids/g VS fed (Tables 4-2 and 4-3).

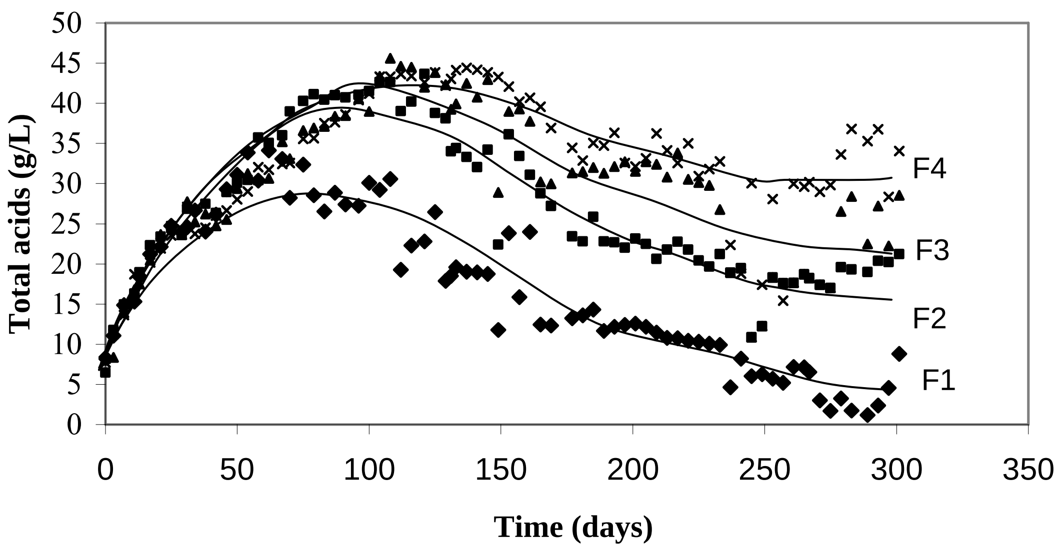


Figure 4-1. Train A total acid concentrations in F1–F4. (Smooth curves are free-hand interpretation of data).

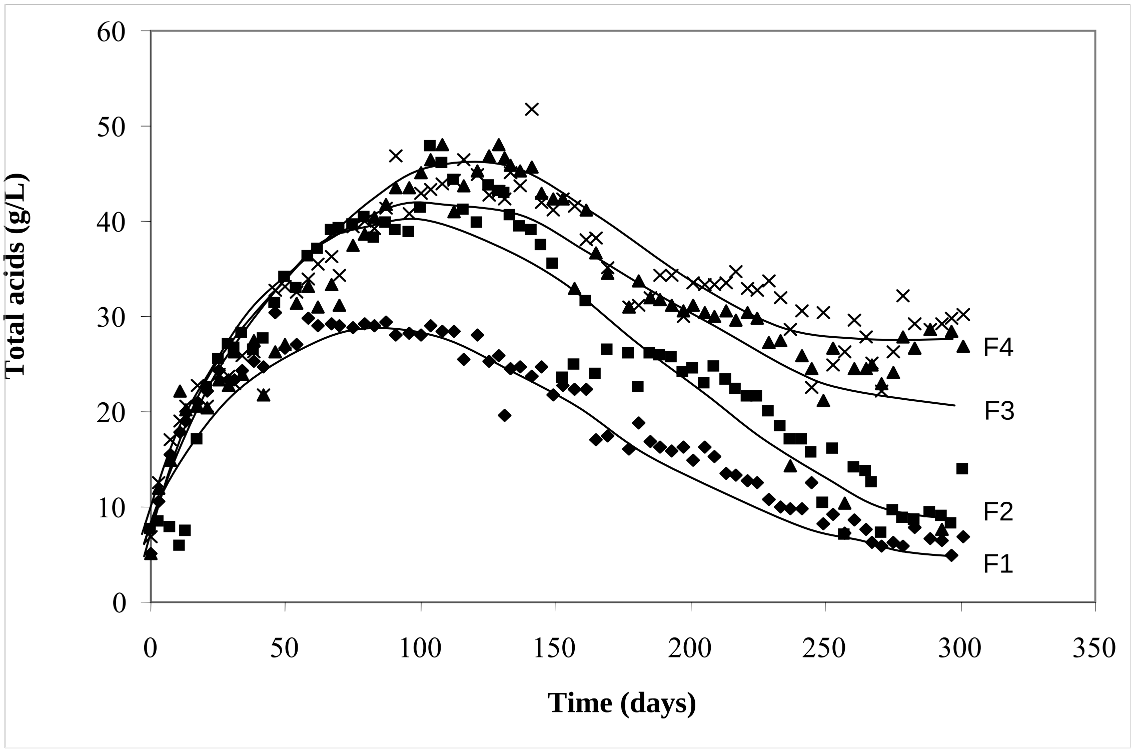


Figure 4-2. Train B total acid concentrations in F1–F4. (Smooth curves are free-hand interpretation of data).

Table 4-2. Fermentation results for Train A

FERMENTATION TRAIN A				
Volatile solids per fermentor (g)	44.0			
Total liquid volume per fermentor (L)	0.514			
Liquid transfer volume per fermentor (L)	0.010			
Temperature (^{o}C)	40			
Frequency of transfer	Every 4 days			
Fermentors	**F1**	**F2**	**F3**	**F4**
Average pH	6.7±0.51	6.5±0.47	6.5±0.49	6.4±0.51
Average total acid productivity(g/(L·d))	0.138	0.158	0.154	0.172
Maximum acid concentration (g/L)	34.2	43.6	45.6	44.4
Average peak acid concentration (g/L)	30.0	40.7	42.2	42.6
Time of maximum acid concentration (d)	46–108	70–129	96–145	96–165
Average VS digested (g/d)	0.123	0.128	0.120	0.120
Yield (g total acids/g VS fed)	0.489	0.560	0.547	0.609
Selectivity (g total acids/g VS digested)	0.579	0.637	0.665	0.741
Conversion (g VS digested/g VS fed)	0.844	0.879	0.821	0.822
Biotic CO_2 productivity (g CO_2/L·d)	0.100	0.079	0.072	0.056
CH_4 productivity (g CH_4/(L·d))	0.0	0.011	0.005	0.004
Mass balance closure (g VS out/g VS in)	1.06	1.10	0.958	1.01

Table 4-3. Fermentation results for Train B

FERMENTATION TRAIN B				
Volatile solids per fermentor (g)	44.0			
Total liquid volume per fermentor (L)	0.514			
Liquid transfer volume per fermentor (L)	0.015			
Temperature (^oC)	40			
Frequency of transfer	Every 4 days			
Fermentors	**F1**	**F2**	**F3**	**F4**
Average pH	6.3±0.54	6.5±0.73	6.3±0.52	6.3±0.53
Average total acid productivity(g/(L·d))	0.188	0.165	0.159	0.162
Maximum acid concentration (g/L)	30.5	47.8	48.0	51.8
Average peak acid concentration (g/L)	28.5	41.8	44.9	43.8
Time of maximum acid concentration (d)	46–121	79–133	91–153	91–157
Average VS digested (g/d)	0.134	0.126	0.111	0.108
Yield (g total acids/g VS fed)	0.669	0.584	0.563	0.574
Selectivity (g total acids/g VS digested)	0.732	0.677	0.739	0.775
Conversion (g VS digested/g VS fed)	0.914	0.863	0.761	0.741
Biotic CO_2 productivity (g CO_2/L·d)	0.061	0.070	0.047	0.040
CH_4 productivity (g CH_4/(L·d))	0.006	0.009	0.009	0.007
Mass balance closure (g VS out/g VS in)	1.11	1.06	0.968	0.920

4.2 Fermentation Trains C and D

The total volatile solid in Trains C and D before fermentation was 21.05 g and the total liquid volume was 384 mL. The fermentations were performed under anaerobic conditions at 40^{o}C. Anaerobic medium (100 mL), 0.4 g of dry nutrients, 0.2 g urea, 30 mL of inocula, and 200 μL of iodoform were added. $CaCO_3$ was not added because the average pH was 9.05 prior to fermentation. To maintain anaerobic conditions, nitrogen from a high-pressure liquid nitrogen cylinder was flushed whenever the fermentors were open to the atmosphere. Batch fermentation was performed by adding 0.2 g of dry nutrients, and 160 μL of iodoform solution every 4 days and 0.1 g urea (if pH was less than 6). $CaCO_3$ (2 g) was added every 4 days when pH was below 7. The gas samples were analyzed to determine the methane content. More iodoform solution (120 μL) was added to fermentors showing high methane content.

After one month, liquid was transferred between the columns followed by the addition of 0.2 g of dry nutrients, 160 μL of iodoform solution every 4 days, and 0.1 g urea (if pH is less than 6). The liquid movement was similar to the procedure for Trains A and B. Liquid volumes of 20 mL and 25 mL were transferred every 4 days for Trains C and D, respectively. These liquid addition rates were estimated to be the amount required to digest all the biomass in all fermentors in approximately 6 months. Train C had leaks on F3 and F4 and Train D also had leaks on F2 and F4.

The results obtained are shown in Figures 4-3 and 4-4. The detailed experimental results are shown in Appendix N (Tables N4I–N4P). The maximum total acid concentration for F1 in Train C was 25.7 g/L and the maximum acid concentration in F2–F4 was ~34 g/L. The maximum total acid concentration in F1 in Train D was 21.8 g/L and the maximum acid concentration in F2–F4 was ~31 g/L. Although there was almost no difference in the total acid concentrations of F2–F4 in both sets, the times at which the maximum peak occurred generally increased from F2 to F4 (Figures 4-3 and 4-4, Tables 4-4 and 4-5). The conversion in each of the reactors in Train C varied from 0.431–0.820 g VS digested/g VS fed, and the yields were in the range 0.255–0.689 g total acids/g VS fed. Conversions and yields in Train D were 0.547–0.969 g VS digested/g VS fed and

0.315–0.808 g total acids/g VS fed (Tables 4-4 and 4-5). Some of the low yields and conversions in Trains C and D were due to the leaks.

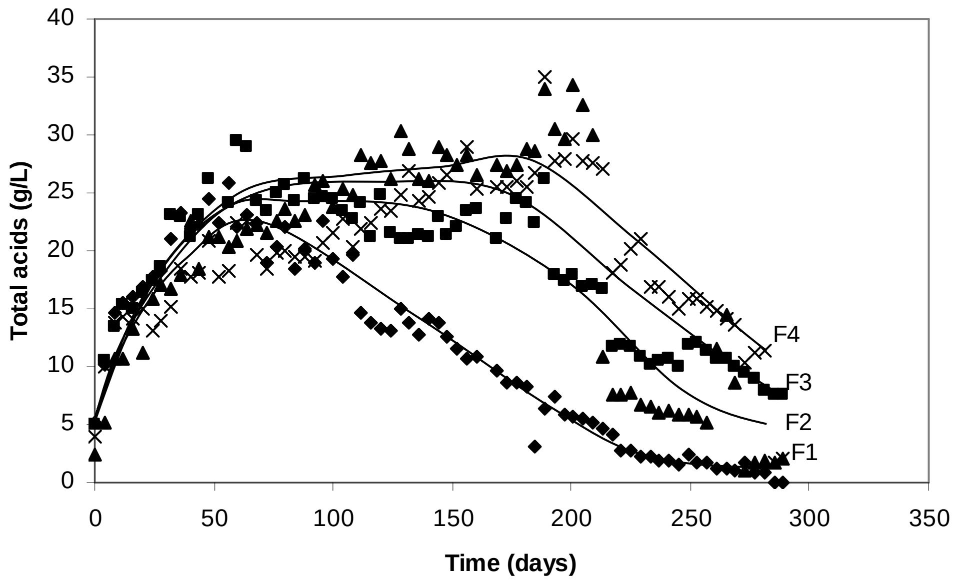


Figure 4-3. Train C total acid concentrations in F1–F4. (Smooth curves are free-hand interpretation of data).

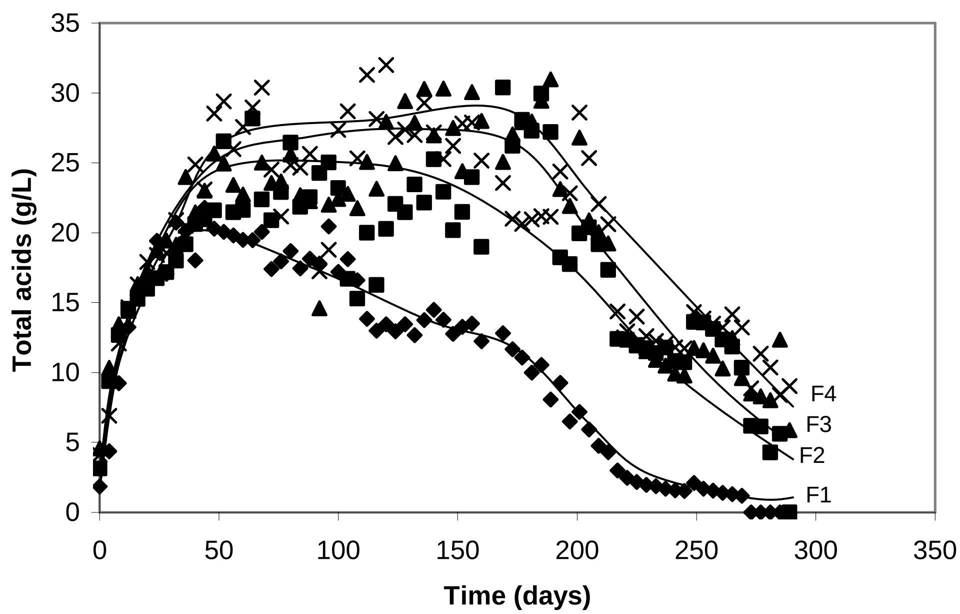


Figure 4-4. Train D total acid concentrations in F1–F4. (Smooth curves are free-hand interpretation of data).

Table 4-4. Fermentation results for Train C

FERMENTATION TRAIN C				
Volatile solids per fermentor (g)	21.05			
Total liquid volume per fermentor (L)	0.378			
Liquid transfer volume per fermentor (L)	0.020			
Temperature (^{o}C)	40			
Frequency of transfer	Every 4 days			
Fermentors	**F1**	**F2**	**F3***	**F4***
Average pH	7.3±0.75	7.1±0.64	6.9±0.63	6.8±0.56
Average total acid productivity(g/(L·d))	0.093	0.093	0.034	0.040
Maximum acid concentration (g/L)	25.8	29.4	34.2	34.9
Average peak acid concentration (g/L)	21.8	24.9	29.0	27.6
Time of maximum acid concentration (d)	32–96	48–120	132–209	156–213
Average VS digested (g/d)	0.060	0.057	0.031	0.034
Yield (g total acids/g VS fed)	0.689	0.690	0.255	0.299
Selectivity (g total acids/g VS digested)	0.840	0.890	0.592	0.646
Conversion (g VS digested/g VS fed)	0.820	0.775	0.431	0.463
Biotic CO_2 productivity (g CO_2/L·d)	0.011	0.007	0.027	0.026
CH_4 productivity (g CH_4/(L·d))	0.014	0.010	0.007	0.005
Mass balance closure (g VS out/g VS in)	1.13	1.12	0.884	0.897

* Leaks

Table 4-5. Fermentation results for Train D

FERMENTATION TRAIN D				
Volatile solids per fermentor (g)	21.05			
Total liquid volume per fermentor (L)	0.378			
Liquid transfer volume per fermentor (L)	0.025			
Temperature (^oC)	40			
Frequency of transfer	Every 4 days			
Fermentors	**F1**	**F2***	**F3**	**F4***
Average pH	7.1±0.73	7.0±0.59	7.2±0.63	7.0±0.57
Average total acid productivity(g/(L·d))	0.154	0.072	0.151	0.060
Maximum acid concentration (g/L)	21.8	30.4	31.0	32.0
Average peak acid concentration (g/L)	18.8	22.2	25.5	26.6
Time of maximum acid concentration (d)	20–108	52–140	48–201	48–156
Average VS digested (g/d)	0.071	0.043	0.061	0.040
Yield (g total acids/g VS fed)	0.808	0.375	0.791	0.315
Selectivity (g total acids/g VS digested)	0.833	0.640	0.943	0.575
Conversion (g VS digested/g VS fed)	0.969	0.587	0.838	0.547
Biotic CO_2 productivity (g CO_2/L·d)	0.025	0.036	0.004	0.032
CH_4 productivity (g CH_4/(L·d))	0.006	0.004	0.005	0.012
Mass balance closure (g VS out/g VS in)	1.14	0.818	1.19	0.886

* Leaks

From the results shown in Tables 4-2 and 4-3, it has been demonstrated that pretreatment and fermentation can be performed in the same unit. High product concentrations ~48 g/L as well as high conversions 0.741 g VS digested/g VS fed (F4, Train B) could be obtained from the same fermentor. Apart from having the benefit of no solids handling issues, the fixed-bed fermentation system can lead to high product concentrations and conversions. From the results, there was no significant difference in maximum product concentration between F2–F4. The difference in F2–F4 was the time at which they peaked. However, the harvested product from F4 begins to decrease when the biomass becomes fully digested. The need for a steady product concentrations calls for a ‘round robin’ system. Although these fermentations were fairly dilute, 85.6 g VS/L liquid (Trains A and B) and 55.7 g VS/L liquid (Trains C and D), the product concentrations from Trains A and B is about 45 g/L and 30 g/L in Trains C and D.

4.3 Modeling Fixed-bed Fermentation

The liquid flow for each fermentor is shown in Figure 4-5. For F1, the liquid input does not have any product whilst liquid output has the product concentration in F1. For the other three fermentors in a set, the input is the product from the previous fermentor and the output is the product concentration in the specific fermentor. The acid accumulation in the fermentor is given by general mass balance equation,

$$\text{Accumulation} = \text{Input} - \text{Output} + \text{Generation} - \text{Consumption} \qquad (2\text{-}23)$$

or

$$\text{Generation} = (\text{Accumulation} + \text{Consumption}) + \text{Ouput} - \text{Input} \qquad (4\text{-}1)$$

Accumulation + Consumption is the grams of acetic acid equivalent produced between consecutive measurements in the fermentor. The Output is the grams of acetic acid equivalents taken from the fermentor on each transfer and Input is the grams of acetic acid equivalent put into the fermentor. In case of F1 in each set, Input = 0.

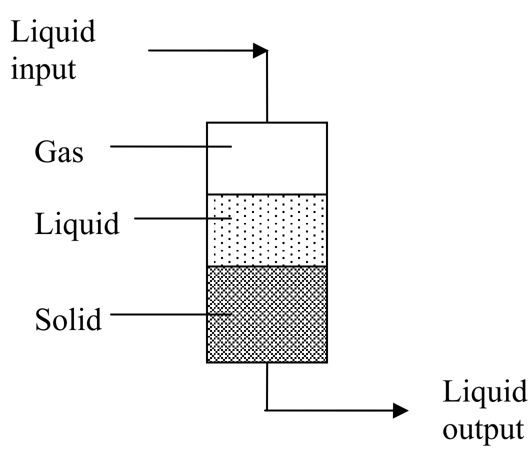


Figure 4-5. Liquid flow in fermentor.

Trains A and B

The parameters obtained from F1 in Train A were used to model the other fermentors in Trains A and B taking the liquid flow in each fermentor into consideration (see Figure 1-8). The experimental data were fit to a parametric equation (Figure 4-6) and the total acetic acid equivalent from the parametric equation was used instead of the actual acetic acid equivalent from the experiment. The mass balance equation (Equation 4-1) was used to determine the acetic acid generation over the entire fermentation period.

The acids generated from F1 in Train A is shown in Figure 4-7. The amounts of acetic acid equivalents generated over time using Equation 4-1 are shown in Figures 4-7 to 4-10. Figures 4-7 to 4-10 show that the entire biomass can be digested in approximately 90 d in F1, 125 d in F2, 140 d in F3 and 150 d in F4. The acid generated was modeled using Equation 2-18.

$$A_e = a + \frac{bt}{1+ct} \tag{2-18}$$

Least squares analysis was used and the following parameters were obtained for Equation 2-18.

$$A_e = 5 + \frac{0.854t}{1+0.0191t} \tag{4-2}$$

where t is in days and A_e is in g/L.

The specific rate was determined from Equation 4-2 and this was fit to Equation 2-20.

$$\hat{r}_{pred} = \frac{e(1-x)^f}{1+g[\phi A_e]^h} \tag{2-20}$$

The parameters for Equation 2-20 were obtained by least-squares fitting of fermentation data from F1 of Train A (Figure 4-7). Equations 4-3 contain parameters for Train A.

$$\hat{r}_{pred} = \frac{0.0243(1-x)^{1.255}}{1+0.0198[\phi A_e]^{1.1}} \qquad \text{(Train A)} \tag{4-3}$$

CPDM parameters (Equation 4-3) were obtained using the least squares analysis to minimize specific rate (g of acetic acid equivalent generated/(time·g VS)) using the mass of acetic acid generated from F1 of Train A. The conversion (*x*) used in the model (Equation 4-3) is defined as the mass of acetic acid equivalent generated (g) per mass of volatile solids (g). During the batch mode of operation, the rate equation (Equation 4-3) was integrated using the explicit method of numerical integration. The mass of acetic acid equivalent taken as a sample during the batch mode was accounted for in the mass balance. During liquid transfer, the mass of acetic acid equivalent in and out of a particular fermentor was taken into consideration. The accounting system provides information on the total mass of acetic acid equivalent in each fermentor during the fermentation. The acetic acid equivalent concentration in the fermentors was determined by dividing the mass of acetic acid equivalents by the volume of liquid in each fermentor. The ratio of total acids to acetic acid equivalents (ø) was used to convert the acetic acid equivalent concentrations back to total acid concentrations. The Matlab program used for modeling is shown in Appendix J and it contains user-friendly comments on how the calculations were done. The predicted total acid concentrations as well as experimental data are shown in Figures 4-11 to 4-18.

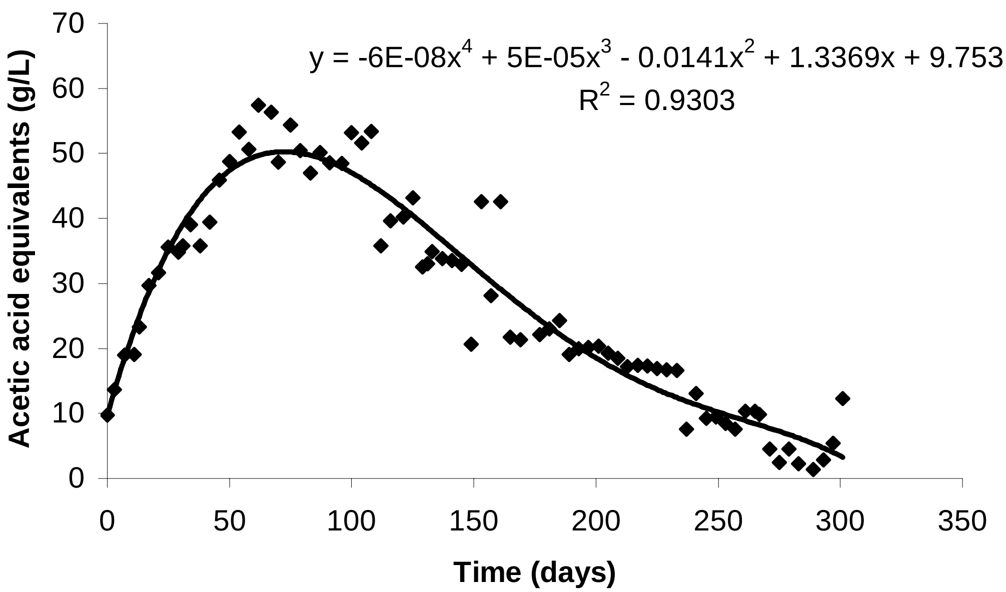


Figure 4-6. Total acetic acid equivalent concentration in F1 for Train A.

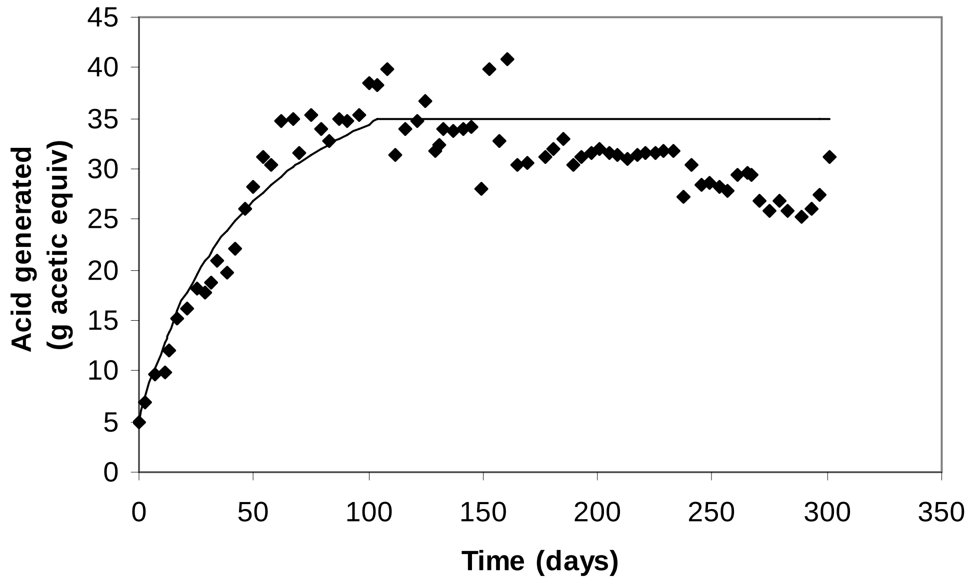


Figure 4-7. Acetic acid equivalents generated in F1 for Train A.

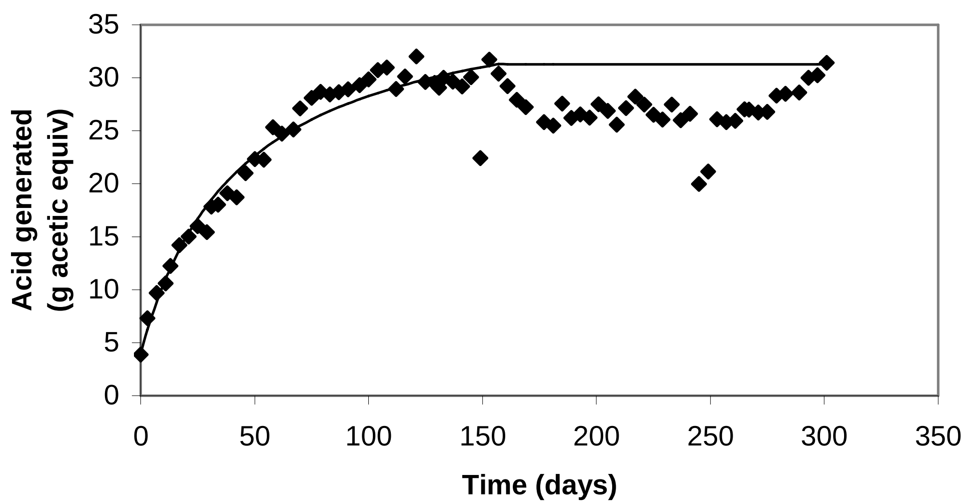


Figure 4-8. Acetic acid equivalents generated in F2 for Train A.

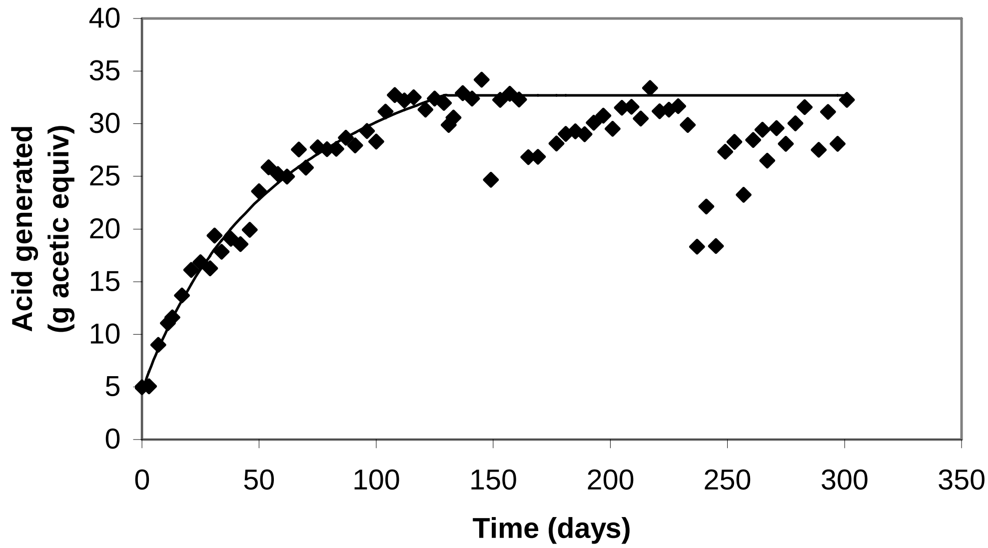


Figure 4-9. Acetic acid equivalents generated in F3 for Train A.

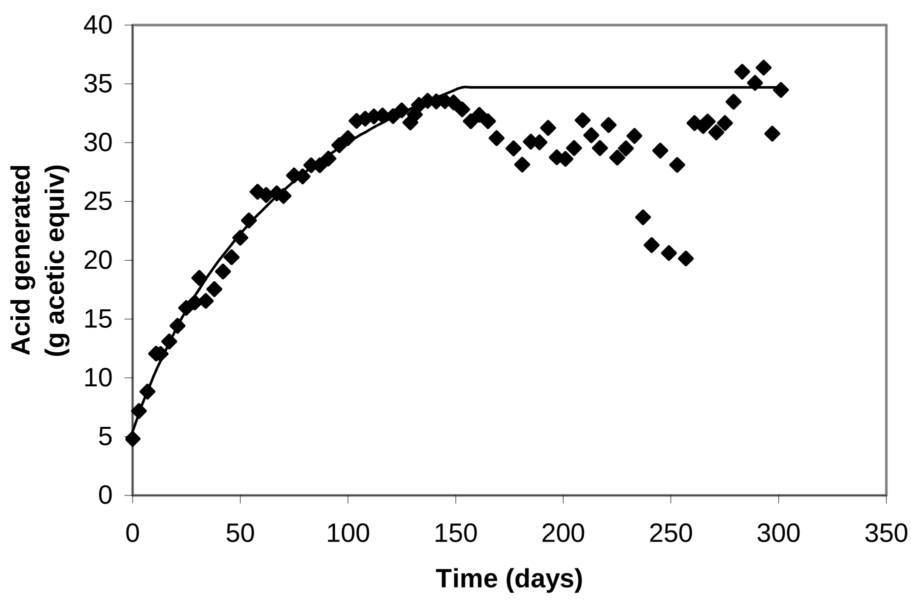


Figure 4-10. Acetic acid equivalents generated in F4 for Train A.

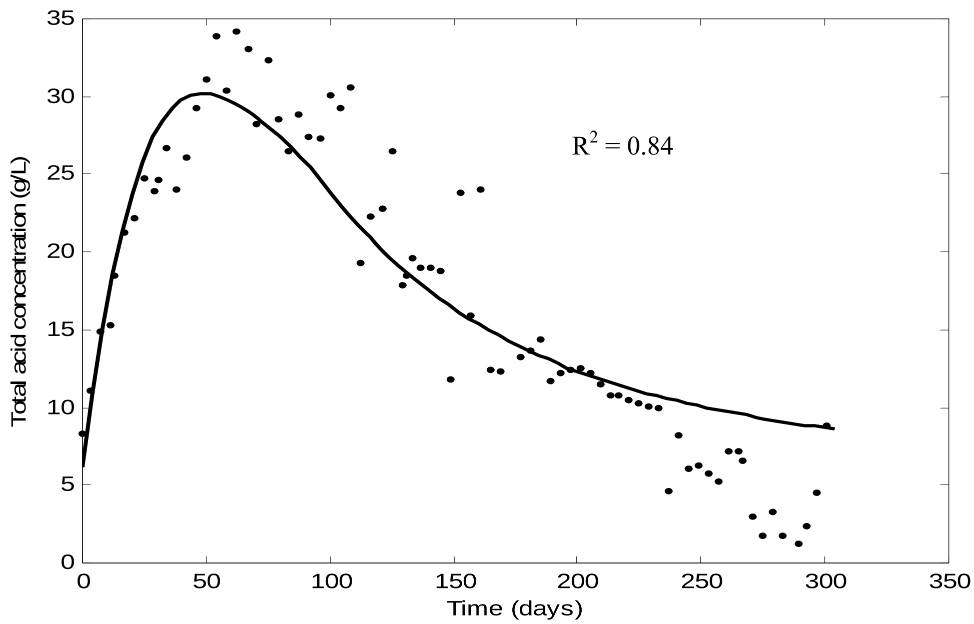


Figure 4-11. Total acid concentrations compared to predictions in F1 of Train A.

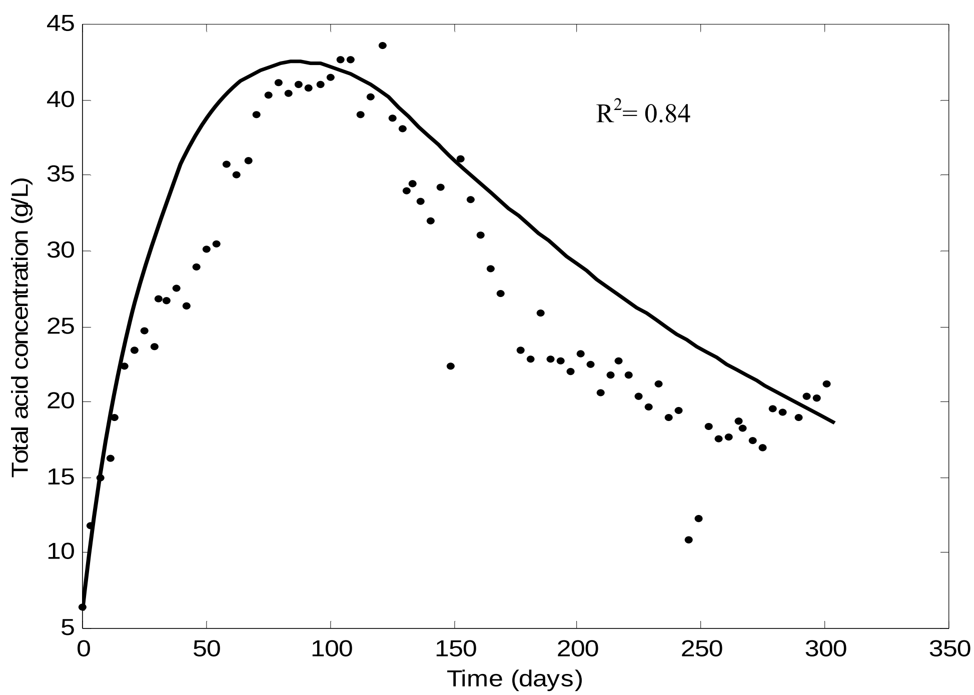


Figure 4-12. Total acid concentrations compared to predictions in F2 of Train A.

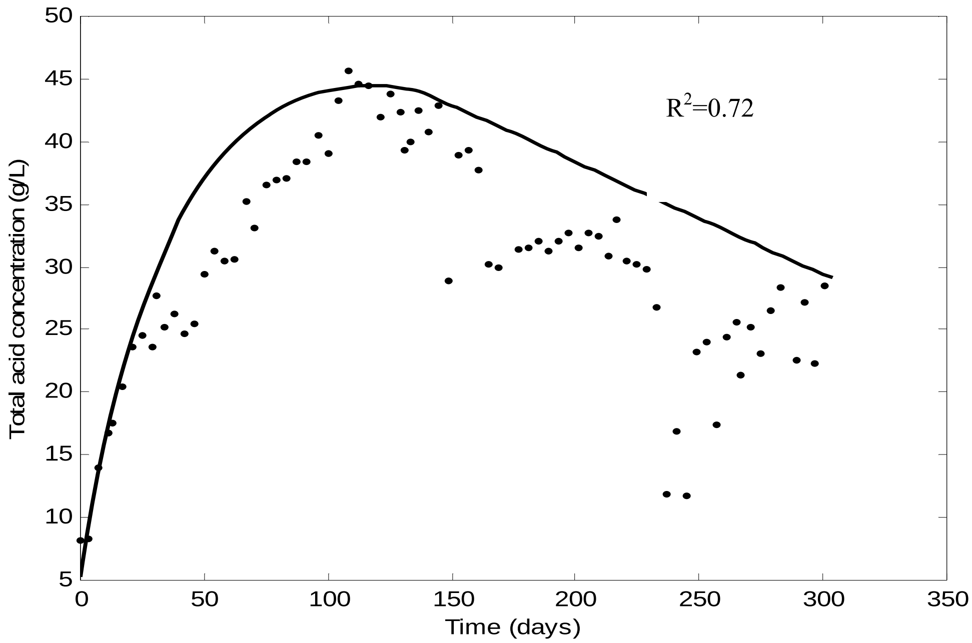


Figure 4-13. Total acid concentrations compared to predictions in F3 of Train A.

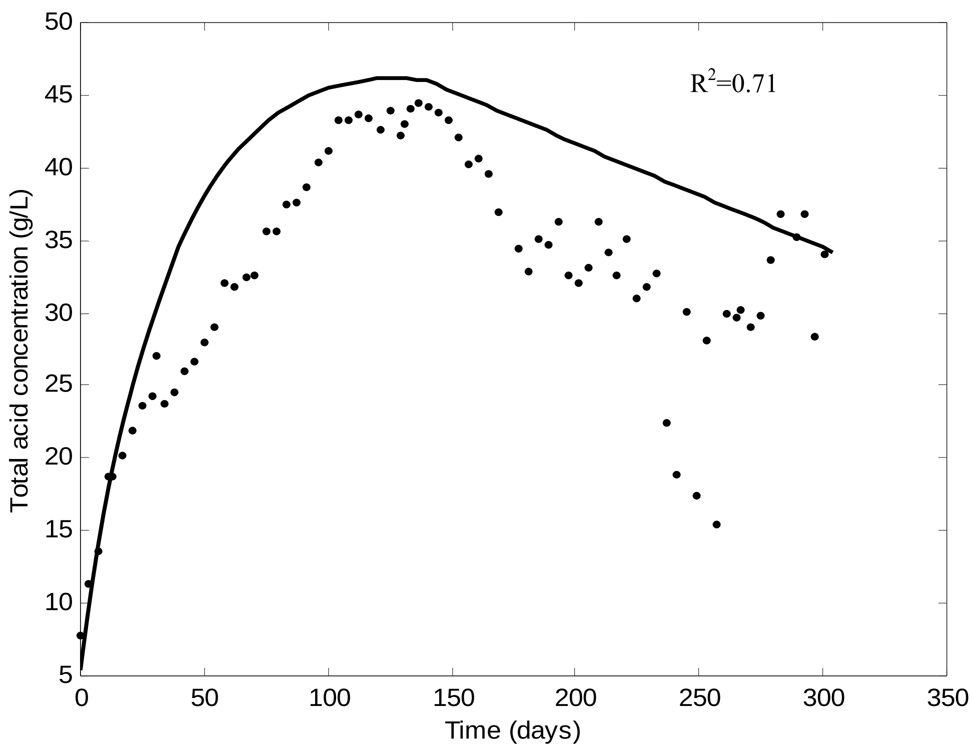


Figure 4-14. Total acid concentrations compared to predictions in F4 of Train A.

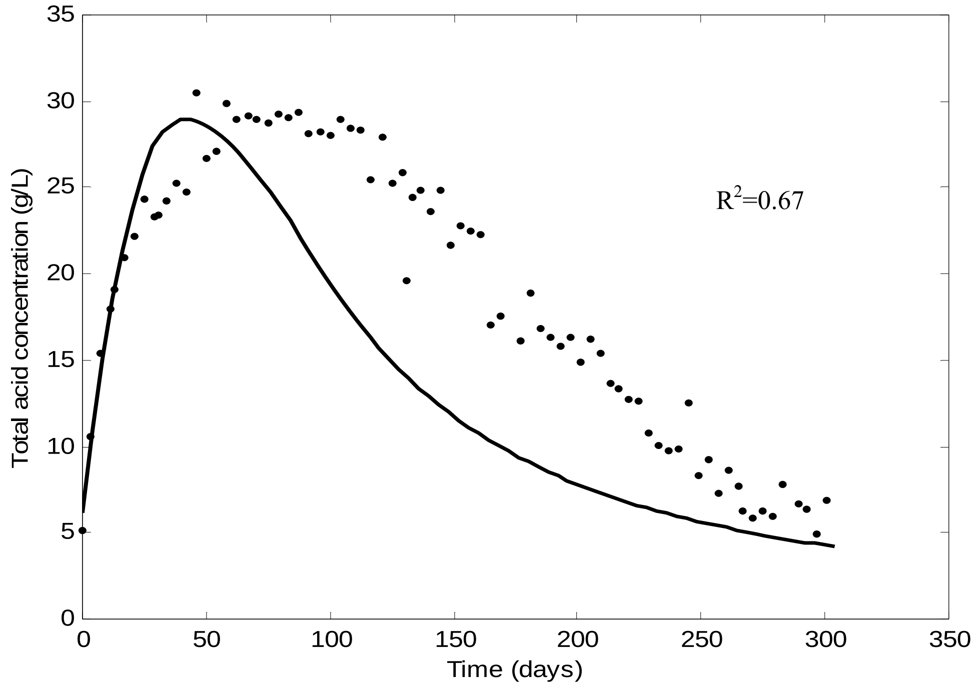


Figure 4-15. Total acid concentrations compared to predictions in F1 of Train B.

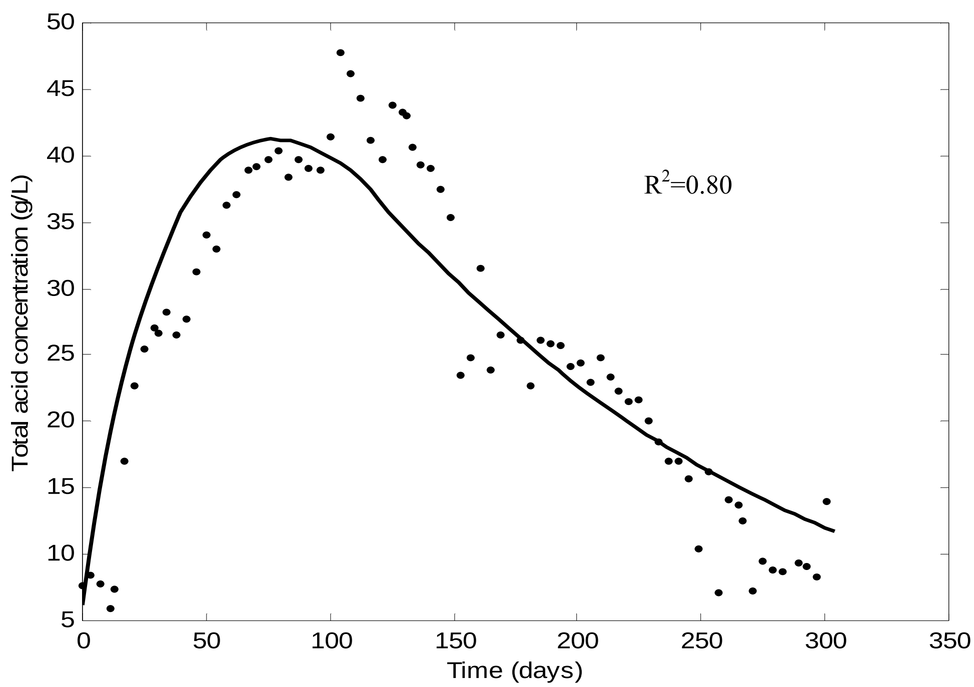


Figure 4-16. Total acid concentrations compared to predictions in F2 of Train B.

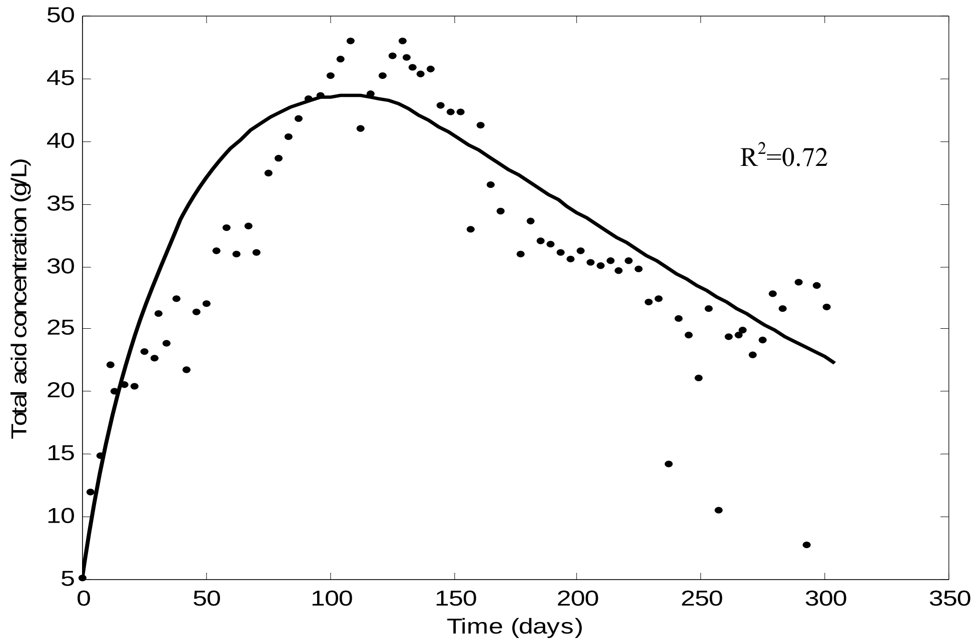


Figure 4-17. Total acid concentrations compared to predictions in F3 of Train B.

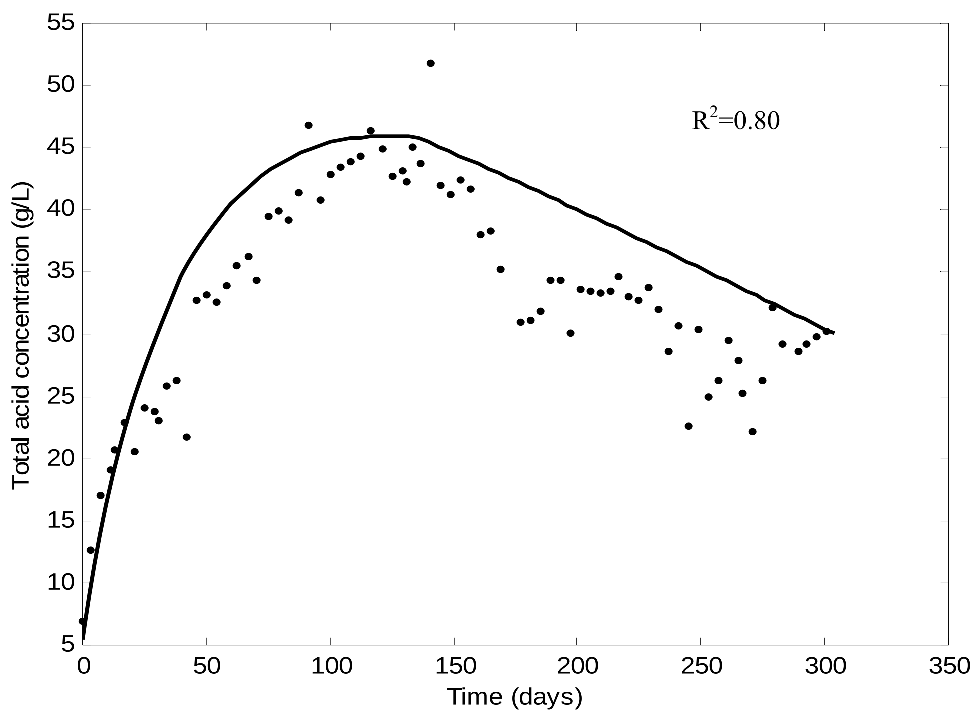


Figure 4-18. Total acid concentrations compared to predictions in F4 of Train B.

The correlation coefficients for the four fermentors in Train A were 0.71–0.84. The same equation was used on to obtain the product concentrations in Train B. The correlation coefficients for the four fermentors in Train B were 0.67–0.80. The predictability of Train B was comparable to that for Train A. The results indicate that data from a single reactor could be used to obtain the product concentrations in the other reactors. A major cause for discrepancy is that the fermentors are not mixed so there is a possibility of channeling and local concentration differences within the fermentors.

Trains C and D

The parameters obtained from F1 in Train C were used to model the other fermentors taking the liquid flow in each fermentor into consideration. The mass of acetic acid equivalent generated over time using Equation 4-1 is shown in Figure 4-19. The CPDM paramenters used for the Matlab program are in Equation 4-4. The Matlab program used for the modeling is shown in Appendix J. In the program, the amounts of acids produced are calculated, and concentrations of the acetic acid equivalents as well as total acid are determined. The predicted total acid concentrations as well as experimental data are shown in Figures 4-20 to 4-27.

$$\hat{r}_{pred} = \frac{0.0288(1-x)^{1.1}}{1+0.0466[\phi A_e]^{1.1}} \qquad \text{(Train C)} \qquad (4\text{-}4)$$

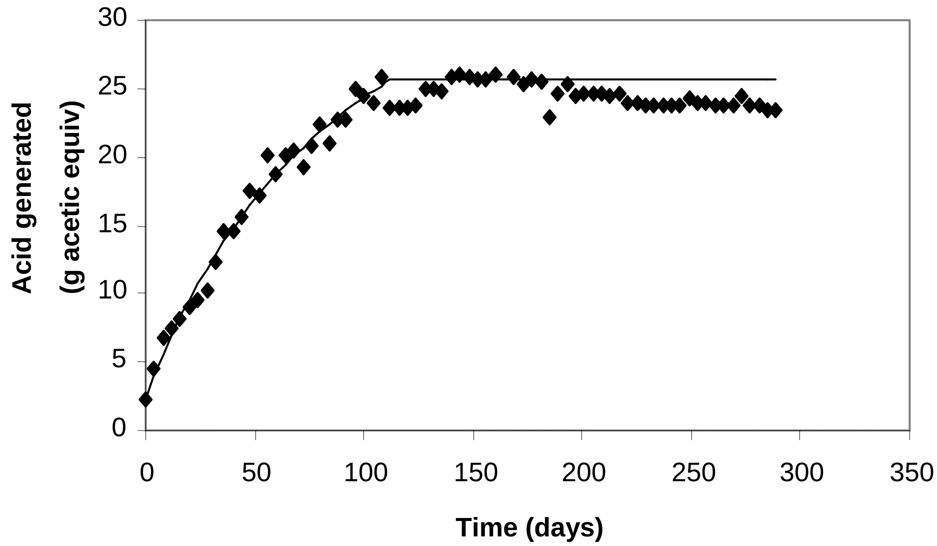


Figure 4-19. Acetic acid equivalents generated in F1 for Train C.

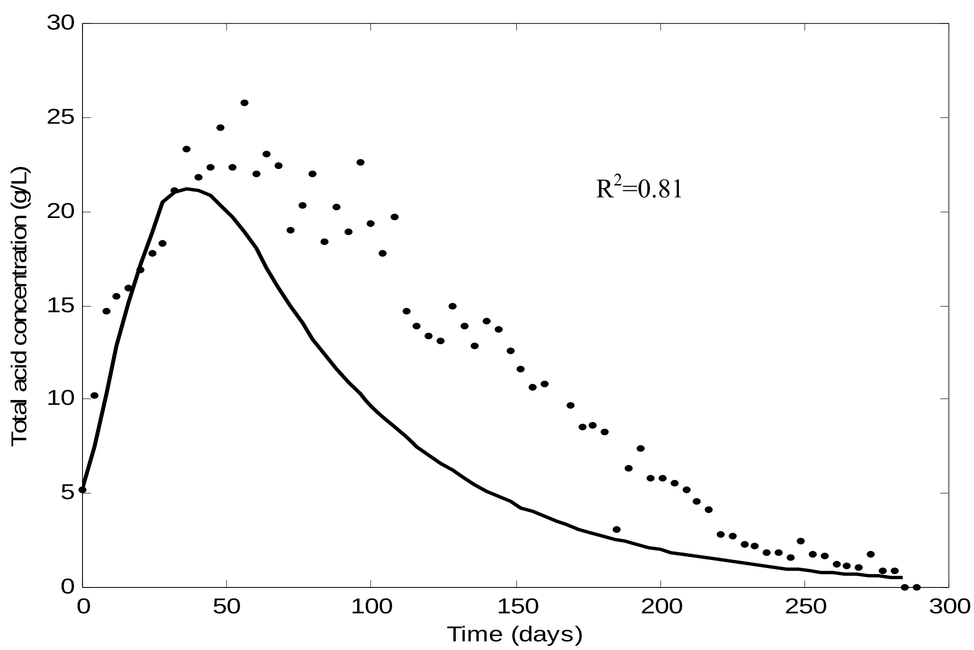


Figure 4-20. Total acid concentrations compared to predictions in F1 of Train C.

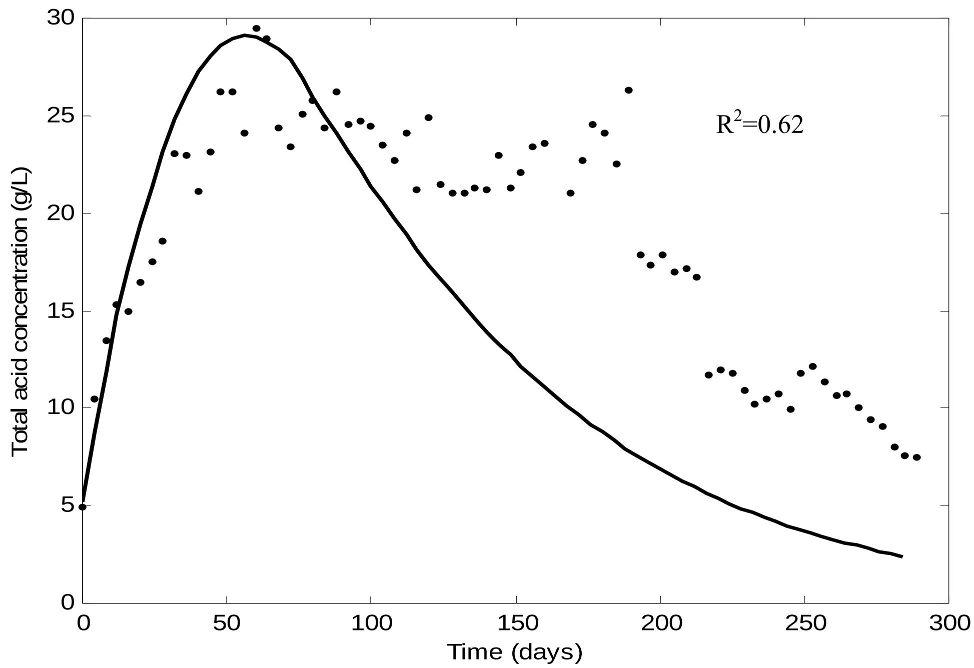


Figure 4-21. Total acid concentrations compared to predictions in F2 of Train C.

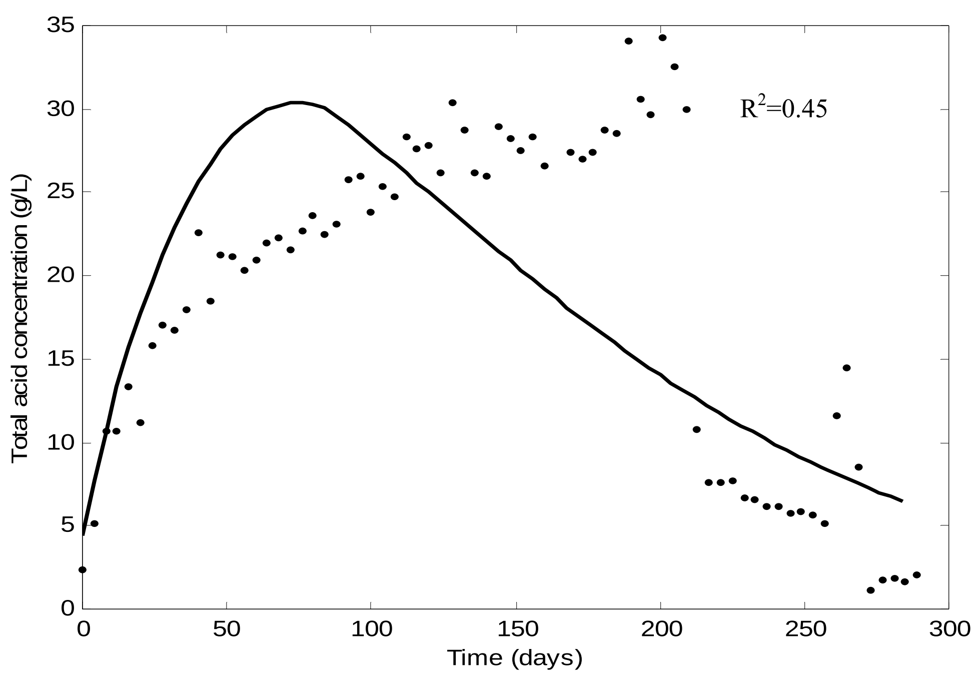


Figure 4-22. Total acid concentrations compared to predictions in F3 of Train C.

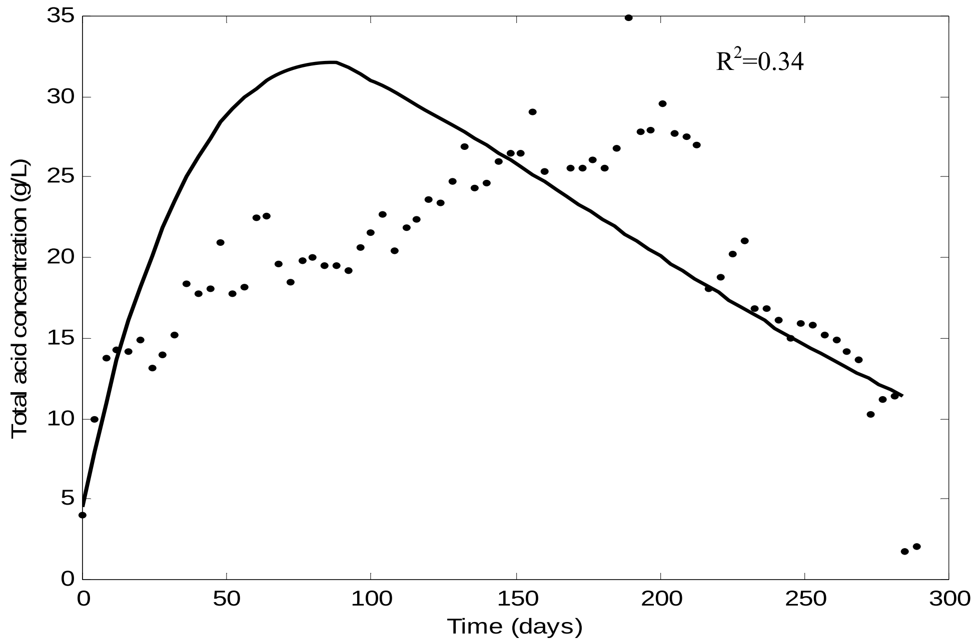


Figure 4-23. Total acid concentrations compared to predictions in F 4 of Train C.

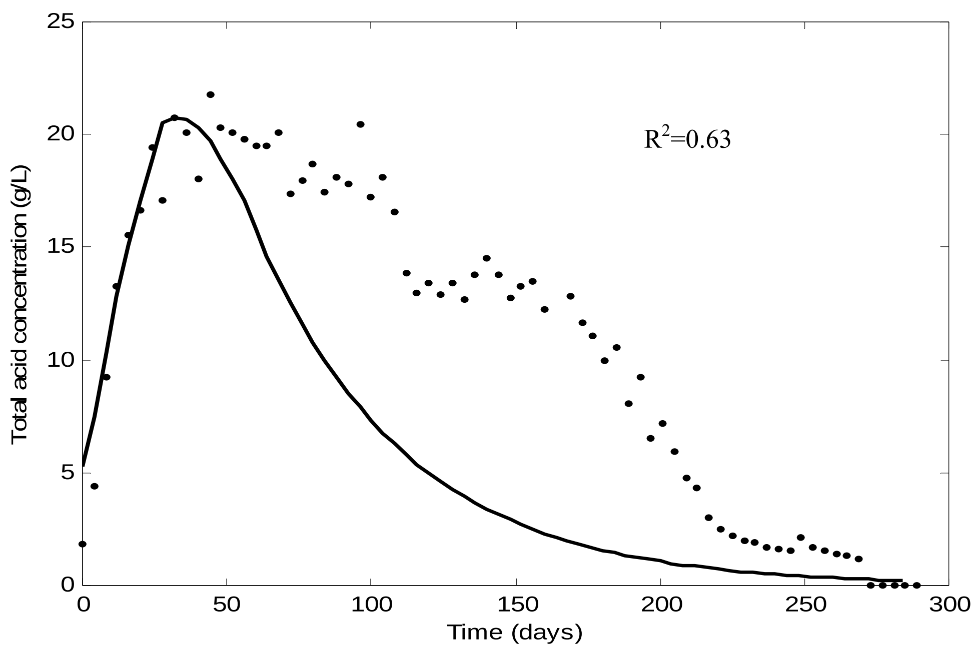


Figure 4-24. Total acid concentrations compared to predictions in F1 of Train D.

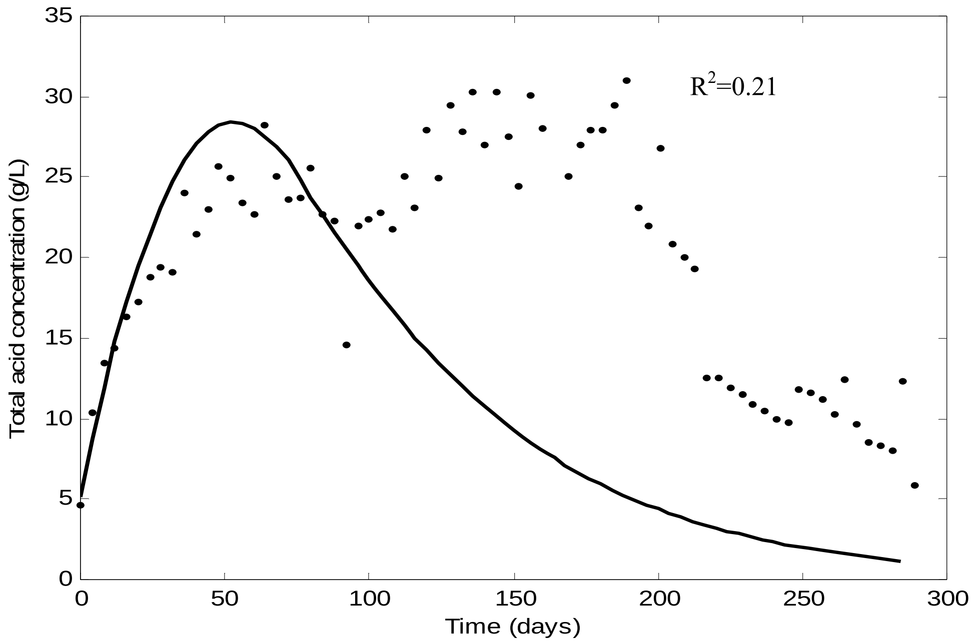


Figure 4-25. Total acid concentrations compared to predictions in F2 of Train D.

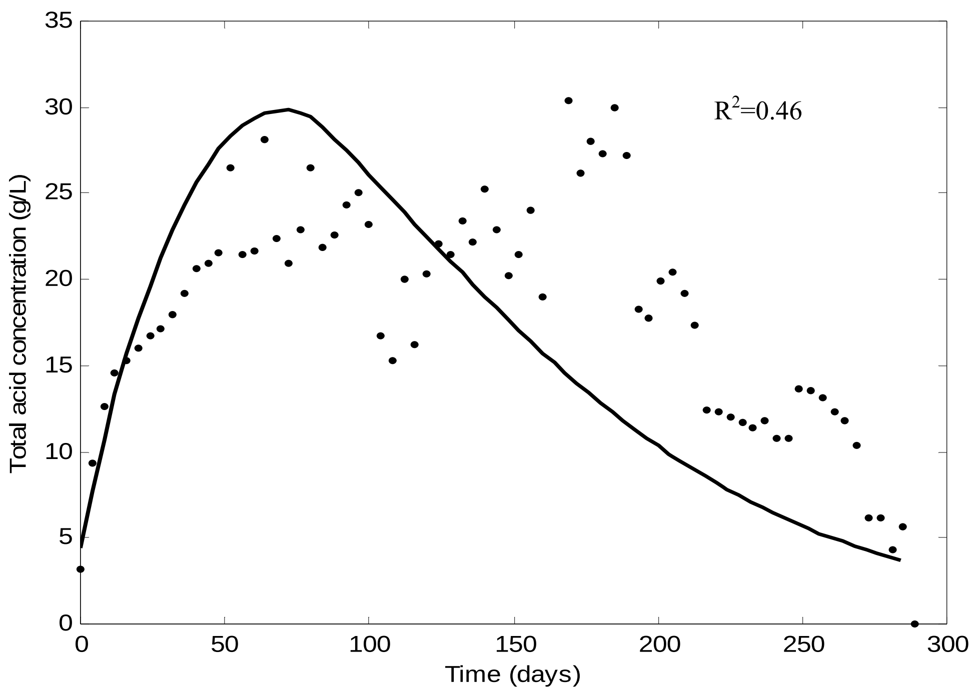


Figure 4-26. Total acid concentrations compared to predictions in F3 of Train D.

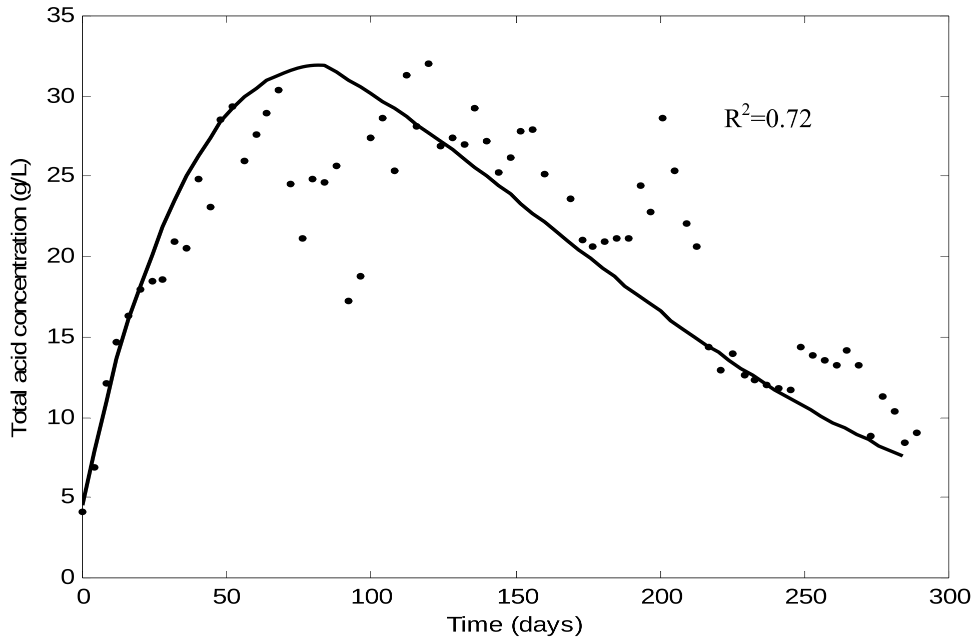


Figure 4-27. Total acid concentrations compared to predictions in F4 of Train D.

Correlation coefficients for the four fermentors in Train C were 0.34–0.81. The same equation was used on to obtain the product concentrations in Train D. Correlation coefficient for the four fermentors in Train D were 0.21–0.72. The predictability of Train C was comparable to results in Train D. The model results from Trains A–D indicate that the CPDM can be extended to predict total acid concentrations in the fermentors. The best correlation was 0.81 for Train C and 0.72 for Train D. Although the correlation for some of the fermentors are very low (0.21), the model gave a pretty good idea about the product concentrations to expect and when to expect them. The predictability of the model can be improved by improving the fermentation data collection process. This will involve preventing channeling, getting uniform product distribution within the fermentors, and preventing leakage.

4.4 The Round Robin System

Steady product concentrations are important in any industrial process; however, using a single fixed-bed fermentation system leads to a variable product concentration. To overcome this, a round robin sytem is proposed as was shown in Materials and Methods. The round robin system can lead to nearly constant product concentrations. Liquid is moved between five fermentors in the round robin system with one fermentor loading and unloading (Figure 2-5). The Matlab program for using CPDM in the round robin system is shown in Appendix K. In the program, the biomass in the fermentor undergoing loading and unloading is pretreated and fermented for 30 d for acids to build up.

During liquid transfer, the mass of acetic acid equivalent in and out of a particular fermentor was taken into consideration. The accounting system provides information on the total mass of acetic acid equivalent in each fermentor during the fermentation. The acetic acid equivalent concentration in the fermentors was determined by dividing the mass of acetic acid equivalents by the volume of liquid in each fermentor. The ratio of total acids to acetic acid equivalents (ø) was used to convert the acetic acid equivalent concentrations back to total acid concentrations. When the conversion in the fermentor (g of acetic acid equivalent/g of VS) is greater than 0.9, the biomass in the fermentor is assumed to be completely digested and the fermentor is switched for another fermentor. The total acid predictions for the round robin system with 44 g of volatile solids per fermentor using liquid transfer volumes of 10 mL, 15 mL, 50 mL, 70 mL, 90 mL and 100 mL every 4 days in a 513 mL initial fermentation liquid volume per fermentor are shown in Figures 4-28 to 4-33. The variation of the frequency of switching fermentors using the different liquid transfer volumes above is shown in Figure 4-34.

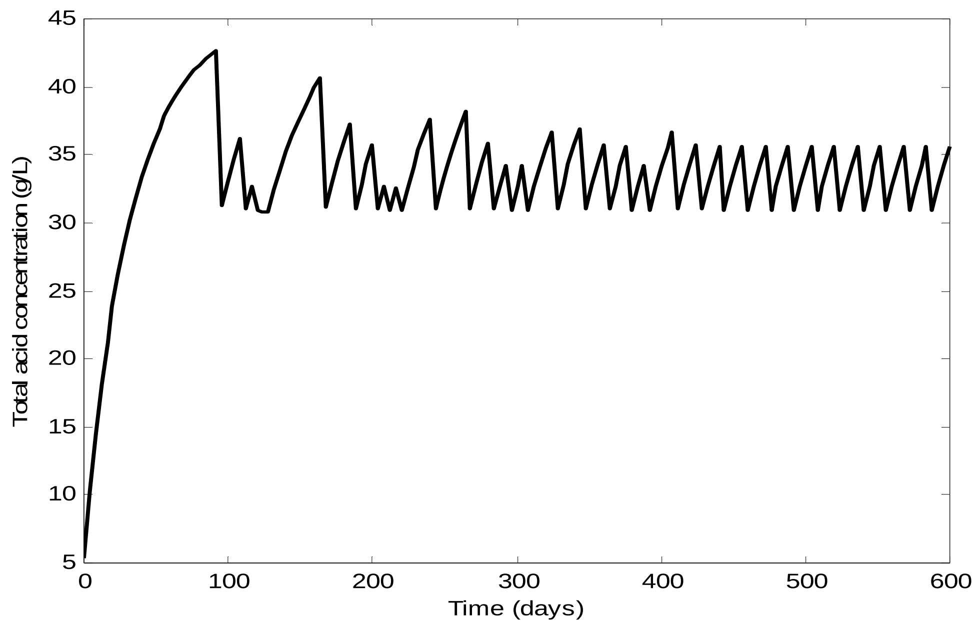


Figure 4-28. Product concentrations in a round robin system (10 mL of liquid transferred every 4 days).

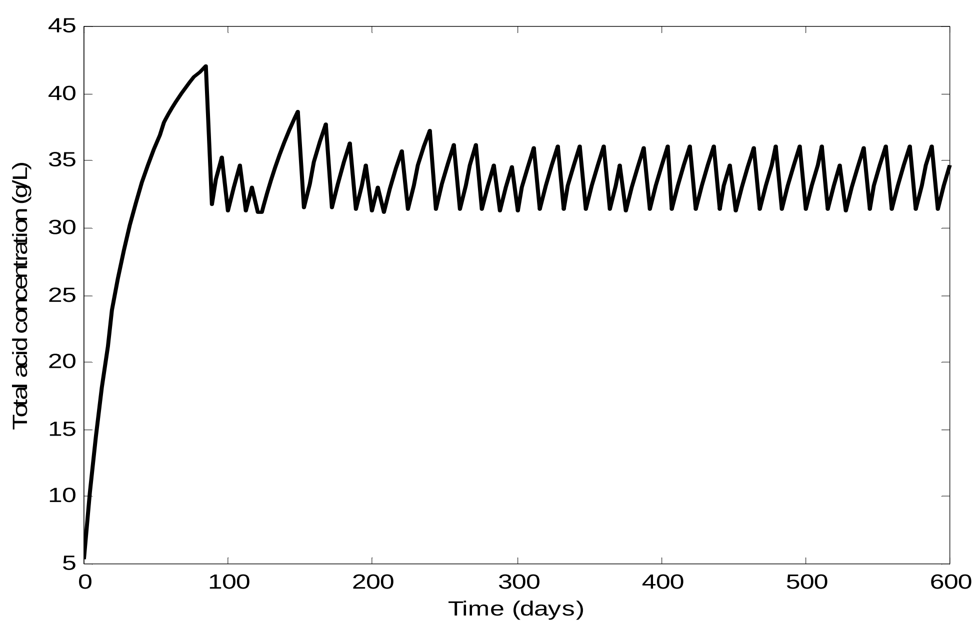


Figure 4-29. Product concentrations in a round robin system (15 mL of liquid transferred every 4 days).

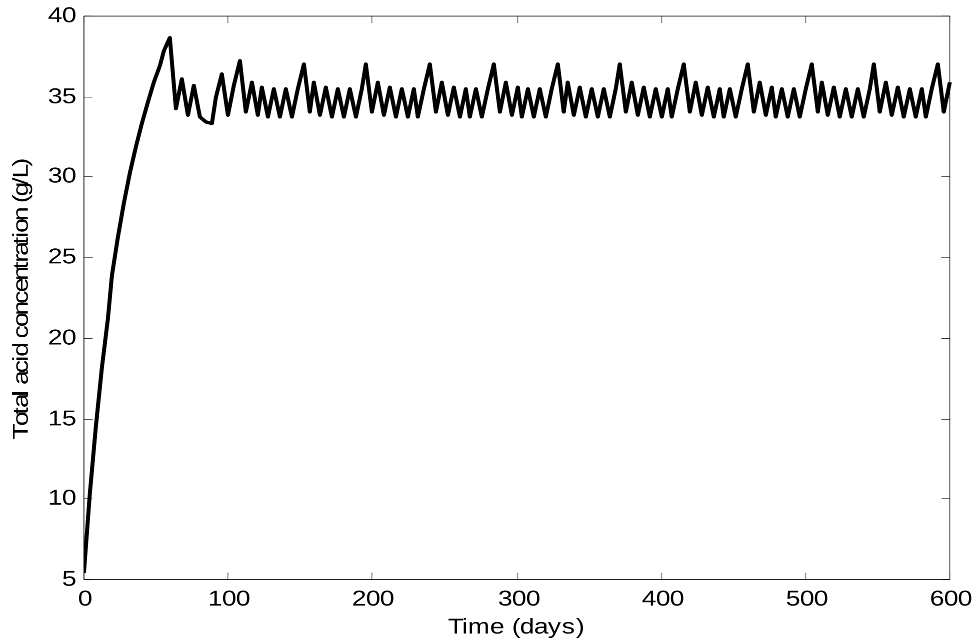


Figure 4-30. Product concentrations in a round robin system (50 mL of liquid transferred every 4 days).

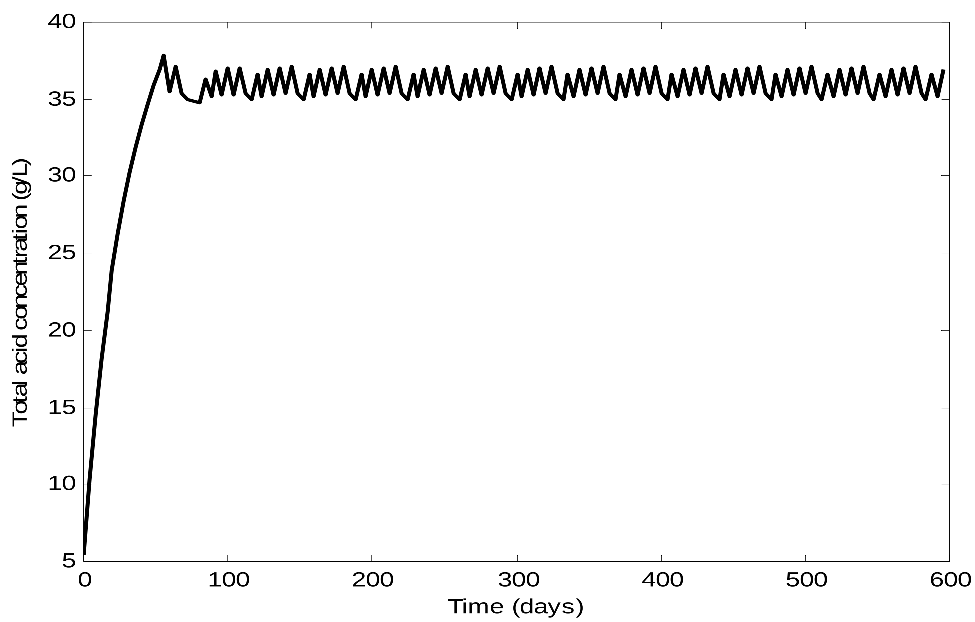


Figure 4-31. Product concentrations in a round robin system (70 mL of liquid transferred every 4 days).

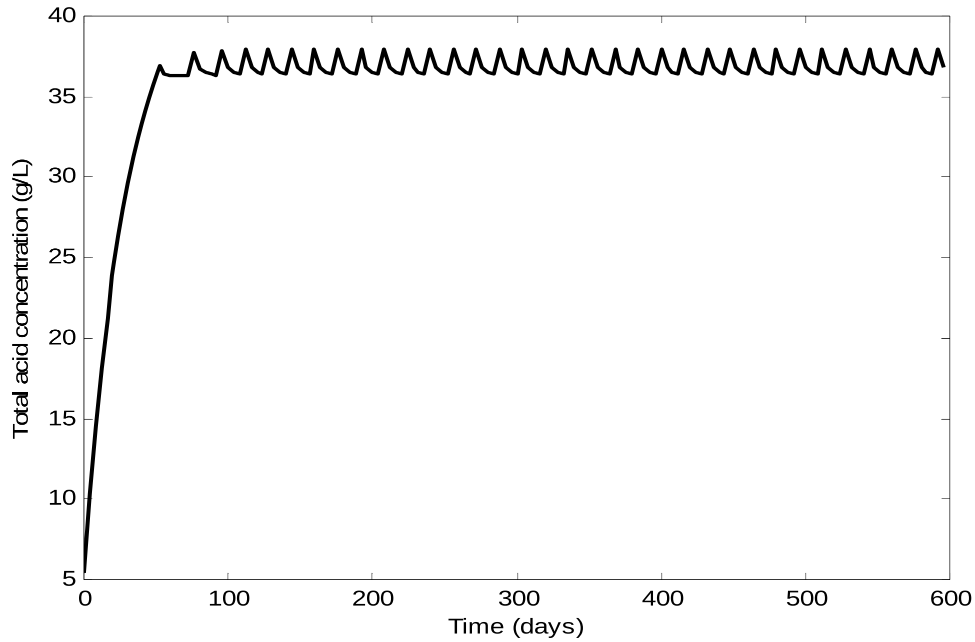


Figure 4-32. Product concentrations in a round robin system (90 mL of liquid transferred every 4 days).

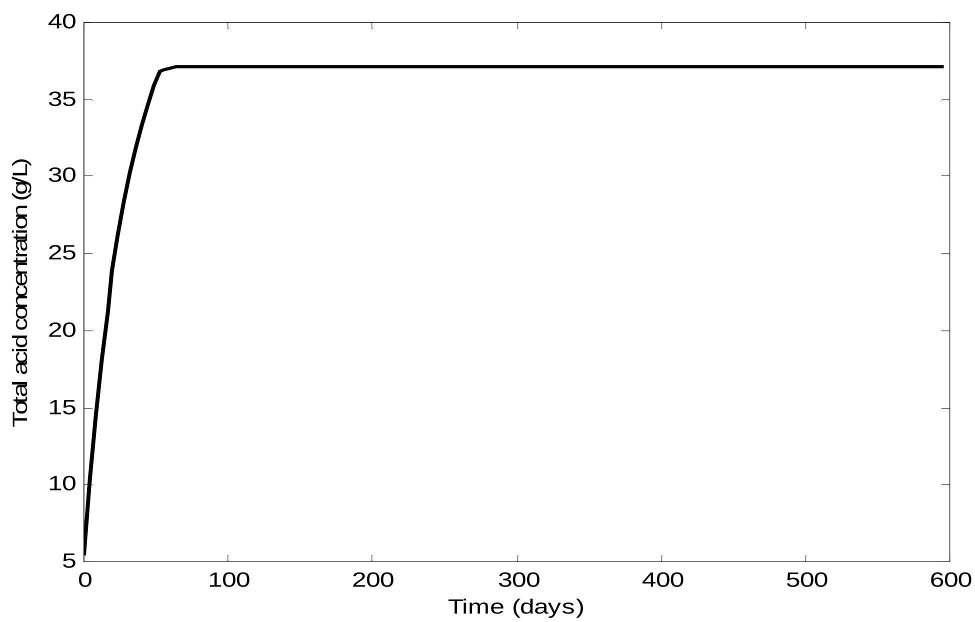


Figure 4-33. Product concentrations in a round robin system (100 mL of liquid transferred every 4 days).

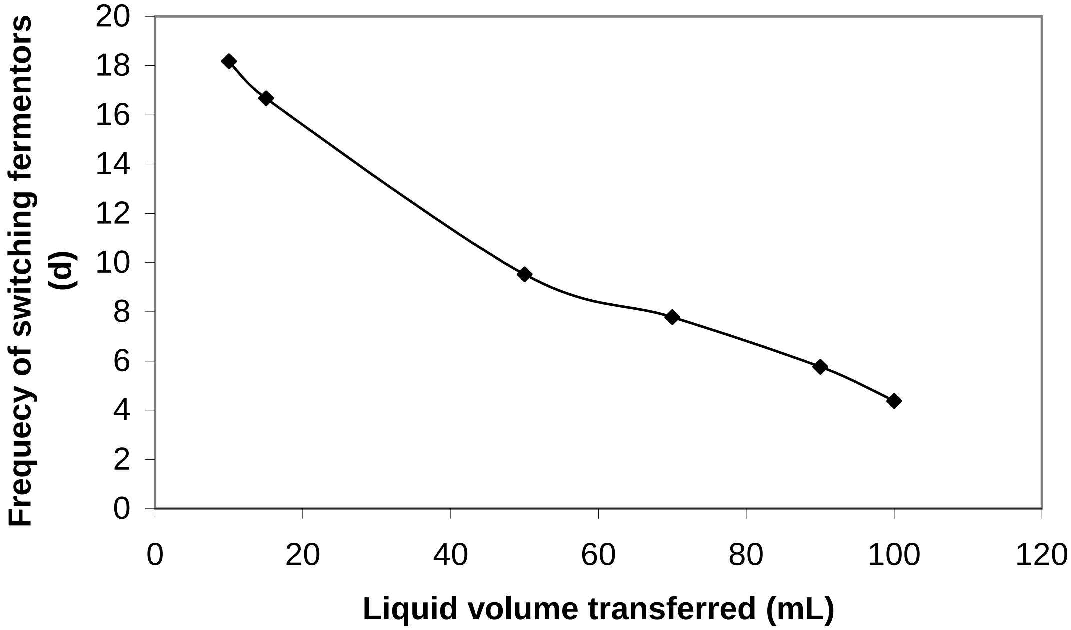


Figure 4-34. Duration of switching fermentors at different liquid transfer volumes.

From Figures 4-28 to 4-33, the noise in the total acid concentration was less as the volume of acid transferred increased. Because a freshly pretreated biomass (44 g volatile solids in 513 mL initial liquid volume) with 30 d batch fermentation can only achieve 30 g/L product concentration, it takes a high liquid transfer volume to get to a steady acid value. From Figure 4-34, there is a general decrease in the number of days it takes to switch fermentors as the quantity of liquid transferred increases. The frequency of switching fermentors is 18 d when only 10 mL of liquid is transferred every 4 d and 4 d when 100 mL of liquid is transferred every 4 d.

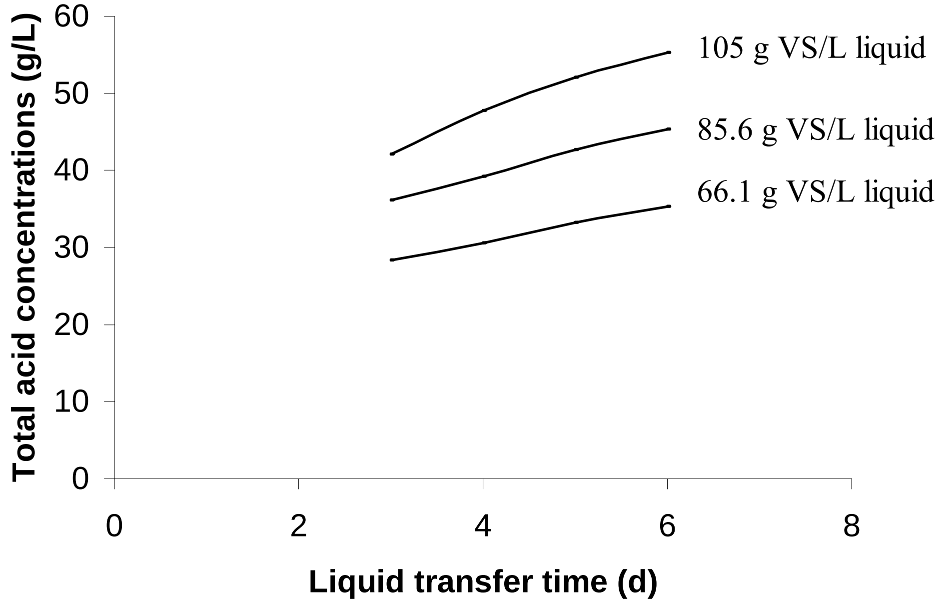


Figure 4-35. Total acid concentrations as a function of liquid transfer time at different concentrations (100 mL transferred in 514 mL initial liquid volume).

The variation of acid concentration with volatile solid concentration and transfer rate in an initial 513 mL liquid volume is shown in Figure 4-35. An increase in the days it takes to do a transfer increases the total acid concentration. This is because the longer it takes to do a transfer, the more the acids build up and the higher the total acid concentration.

The dependence of total acid concentration on liquid volume transferred at various volatile solid concentrations is shown in Figure 4-36. It shows that the total acid concentration does not vary with the liquid volume transferred. However, small-volume liquid transfers every 4 days (10 mL, 15 mL) have more noise in the data as shown above.

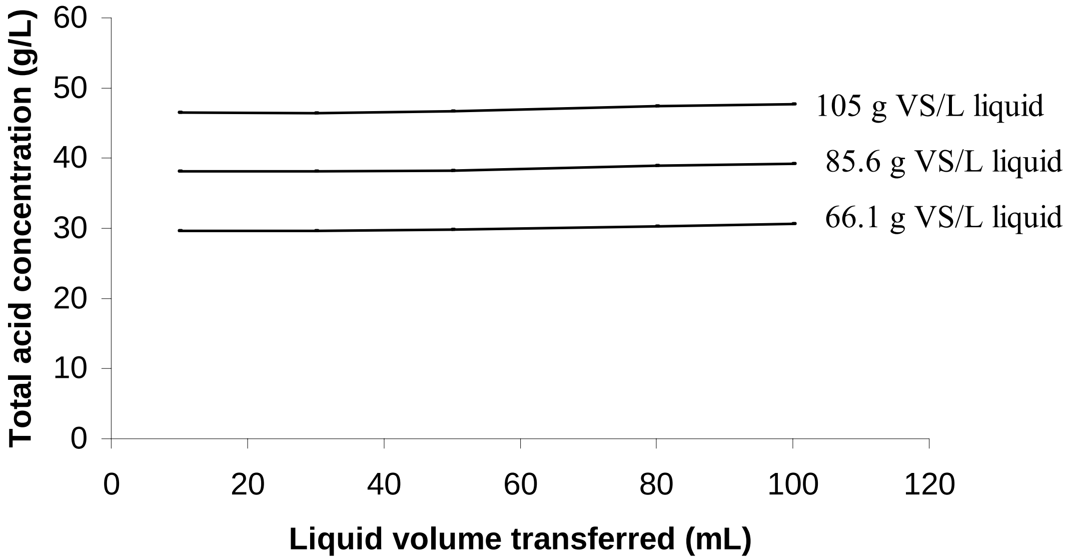


Figure 4-36. Total acid concentrations as a function of liquid volume transferred (4-d transfer).

4.5 Conclusion

For the first time, it has been demonstrated that pretreatment and fermentation can be performed in the same fermentor. Fermentation Trains A and B have high total acid concentrations (45.6 g/L for Train A and 48 g/L for Train B), which is higher than the total acid concentrations in the countercurrent fermentation system. The conversion in each of the reactors in Train A varied from 0.821–0.879 g VS digested/g VS fed, the yield was 0.489–0.609 g total acids/g VS fed, and the selectivity was 0.579–0.741 g total acids/g VS digested, respectively. The conversion, yield, and selectivity in Train B were 0.741–0.914 g VS digested/g VS fed, 0.563–0.669 g total acids/g VS fed, and 0.677–0.775 g total acids/g VS digested, respectively. The conversion, yield, and selectivity in Train C were 0.431–0.820 g VS digested/g VS fed, 0.255–0.69 g total acids/g VS fed, and 0.592–0.840 g total acids/g VS digested, respectively. The conversion, yield, and selectivity in Train D were 0.547–0.97 g VS digested/g VS fed, 0.315–0.808 g total acids/g VS fed, and 0.575–0.833 g total acids/g VS digested, respectively.

The extension of the CPDM model to predict acid concentrations in the fixed-bed fermentation system produced satisfactory results with R^2 of 0.67–0.84 in Trains A and B. For Trains C and D, the R^2 was 0.21–0.81. The model was extended to the round robin system to predict steady total acid concentrations. The noise in the predicted total acid concentrations was higher at lower liquid transfer rates compared to high liquid transfer rates. The total acid concentration increased with increasing duration of transfer at various volatile solid concentrations.

CHAPTER V
USING ASH TO PRETREAT BIOMASS

5.1 Poplar Wood

In this study, ash from poplar wood was used to pretreat poplar wood. Poplar wood was ashed at 575°C overnight to obtain the ash. Initial titrations demonstrated that it takes 4 times the weight of ash to get the equivalent alkalinity as lime ($Ca(OH)_2$). Poplar wood was sieved through a 40-mesh sieve. The sample (2.5 g) was mixed with 100 mL of water in a 125-mL flask. Ash (1 g) and 0.25 g of $Ca(OH)_2$ were added to the flasks respectively. Experiments were performed in duplicate at time intervals of 0, 1, 2, 3, 4, and 6 weeks. All the samples were pretreated at 55°C for 6 weeks. The equipment setup is shown in Figure 5-1.

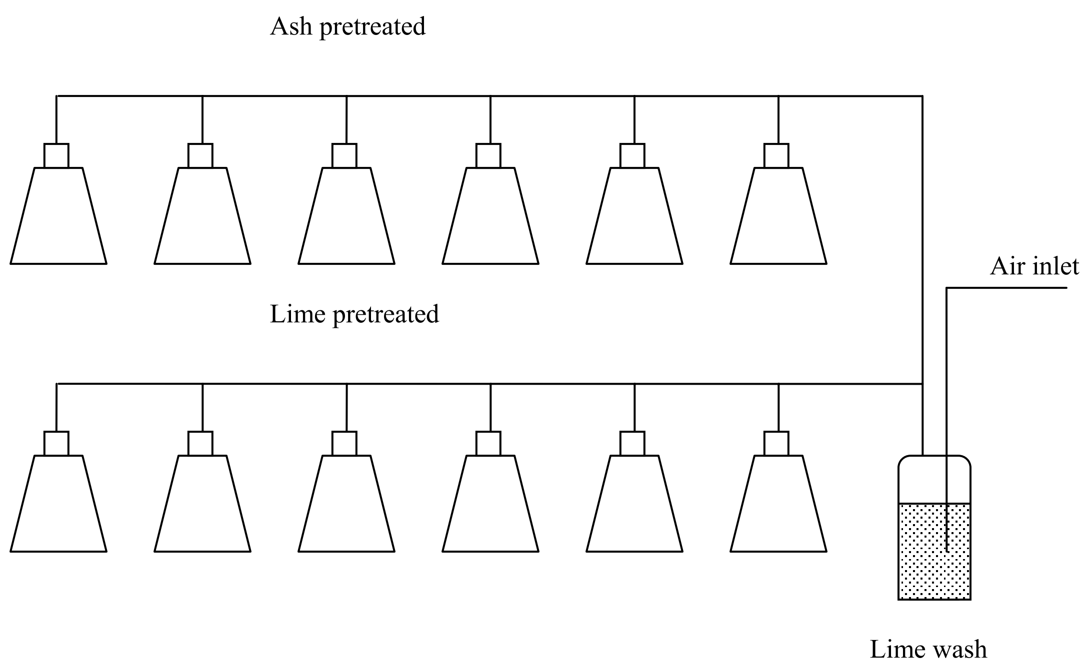


Figure 5-1. Ash and lime pretreatment setup.

The flasks were covered with a rubber septum having two openings at the top, one for air inlet and the other for the air outlet. The inlet air was scrubbed in a lime-wash to remove CO_2. The air flow was adjusted such that all the flasks had air bubbles going through them. The setup was put in an incubator with a shaker and a temperature controller to maintain the temperature at 55°C. When samples were taken out, 30 mL was titrated with 0.1-N HCl to a pH of 7 to determine the equivalent OH^- ions. The volume of 0.1-N HCl titrated is a measure of how much alkalinity was consumed in the ash and lime. The volume of acids titrated is shown in Figure 5-2. The experimental data are shown in Appendix N (Table N5A).

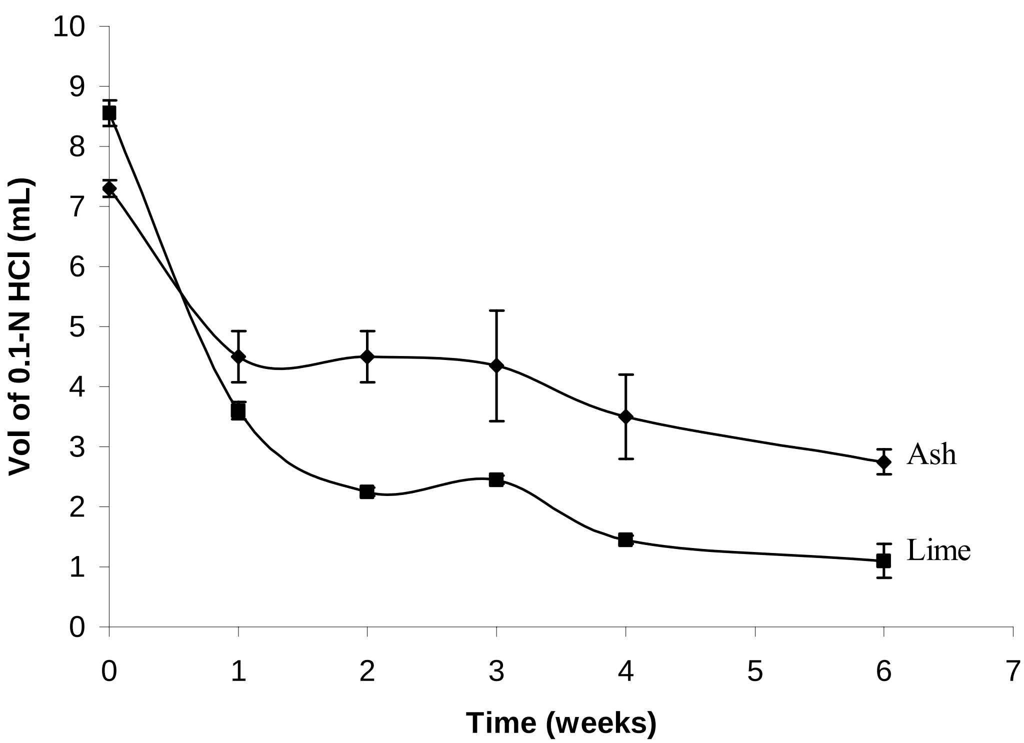


Figure 5-2. Titration of poplar wood treated with lime and poplar wood ash per 30 mL of pretreatment mixture.

From Figure 5-2, the volume of 0.1-N HCl required to neutralize ash-treated poplar wood reduced from 7.3 mL to 2.75 mL. This indicates that poplar wood ash had OH^- ions that were consumed during pretreatment. The volume of 0.1-N HCl required to neutralize lime-treated poplar wood decreased from 8.55 mL to 1.1 mL. There was more reduction in the acid equivalent of lime compared to ash.

The pretreated samples were prepared for both lignin analysis and 3-d digestibility studies. The pretreated samples were neutralized with acetic acid, a mild organic acid. The resulting residue was washed several times until the water became very clear. The residue (poplar wood) was air-dried in the 45°C oven so as not to affect the crystallinity of the biomass. Lignin analysis as well as 3-d digestibility study were performed on the pretreated biomass. Lignin analysis was performed using the NREL procedure described in Appendix L. The total percentage lignin in the substrates is shown in Figure 5-3. The experimental data are shown in Appendix N (Table N5B).

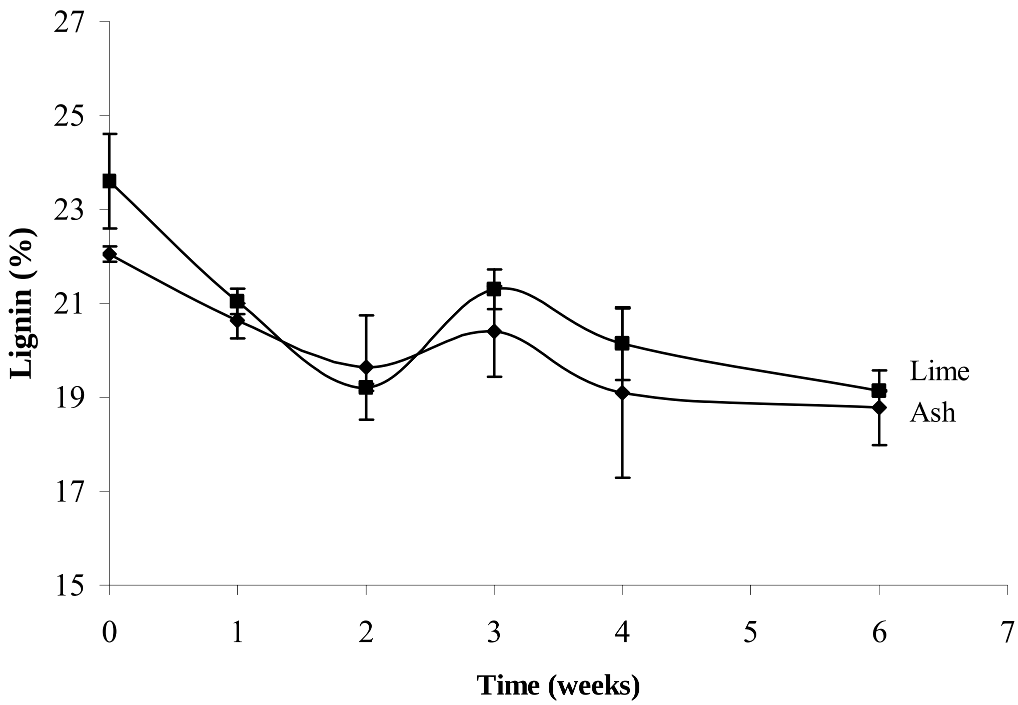


Figure 5-3. Lignin content with time for ash and lime pretreatment.

The percentage lignin in ash-treated poplar wood decreased from 22.1% to 18.8% and that of lime-treated poplar wood decreased from 23.6% to 19.1%. This shows that both ash and lime reduced the lignin content of poplar wood. Three-day digestibility was performed on pretreated biomass by determining the total sugar yield in the biomass. Pretreated poplar wood (0.1 g) was added to 1 mL of 1-M citrate buffer, 0.6 mL of sodium azide, 1 mL of cellulase equivalent to 20 FPU/g biomass, 1 mL of cellobiose 40 CBU/g biomass, and 16 mL of deionized water. The DNS method (Appendix M) was used to analyze the sugars produced. A calibration curve was generated using glucose at concentrations of 0, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL (see Figure 5-4).

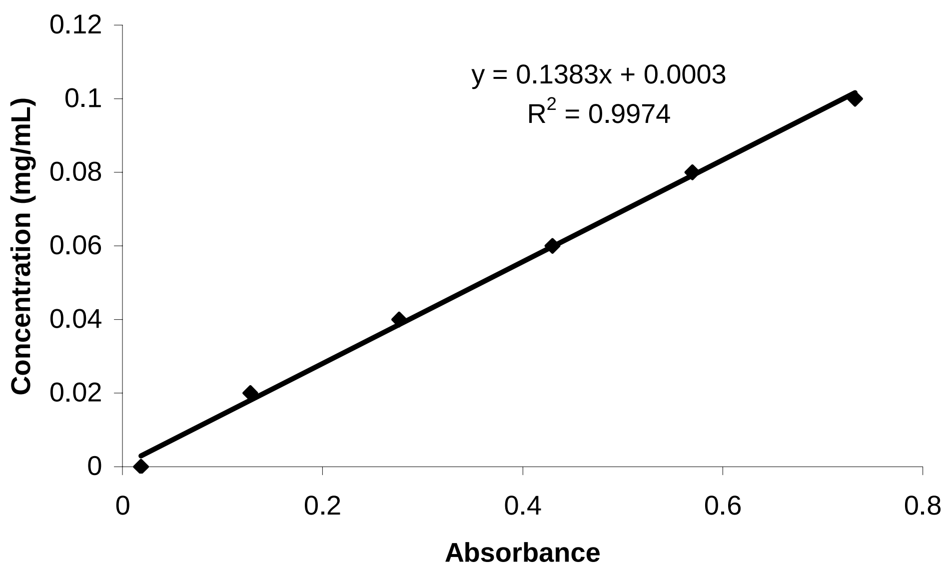


Figure 5-4. Calibration curve using a glucose standard.

The calibration curve was used to determine the equivalent glucose concentration in the hydrolyzed samples. The sugar yields for both lime and ash pretreated biomass are shown in Figure 5-5. The experimental data are shown in Appendix N (Table N5C).

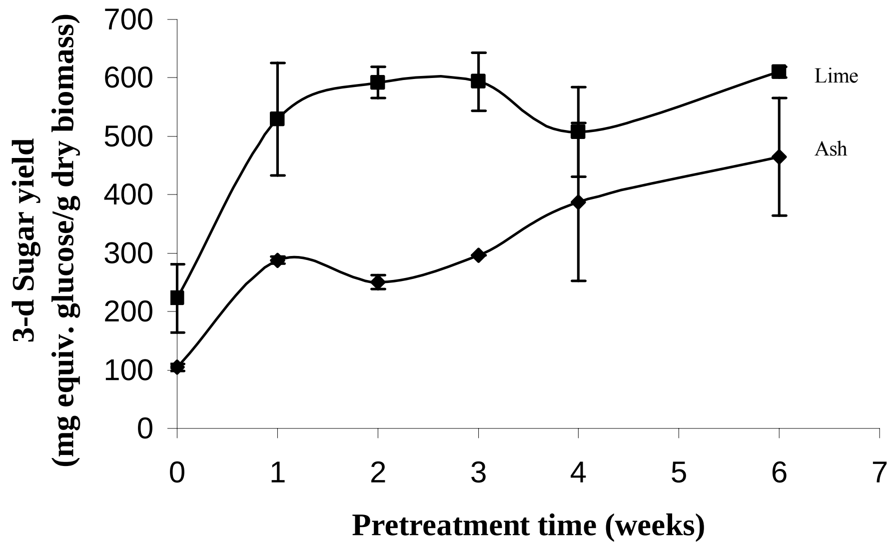


Figure 5-5. Sugar yield for ash- and lime-pretreated poplar wood at 20 FPU/g biomass.

After 6 weeks of pretreatment, the sugar yield for ash-pretreated poplar wood increased from 104 mg equivalent glucose/g dry biomass to 464 mg equivalent glucose/g dry biomass. After 6 weeks of pretreatment, lime-pretreated poplar wood increased from 222 mg equivalent glucose/g dry biomass to 609 mg equivalent glucose/g dry biomass. The sugar yield increased by 360 mg equivalent glucose/g dry biomass in ash-pretreated biomass and 390 mg equivalent glucose/g dry biomass in lime-pretreated poplar wood. This indicates that poplar wood ash is an effective pretreatment agent.

5.2 Bagasse

Ash from sugarcane bagasse fermentation residue was explored. Raw bagasse was pretreated just like the poplar wood, except that 0.4 g of $Ca(OH)_2$ and 1.6 g of ash were used at 50°C. The ash was obtained by putting bagasse fermentation residue in a 550°C oven overnight. Following the pretreatment, the alkalinity of the pretreatment chemicals (ash or $Ca(OH)_2$) and 3-d sugar digestibility was determined. Acid equivalents are shown in Figure 5-6. From the figure, it is clear that ash from bagasse fermentation residues does not have alkalinity.

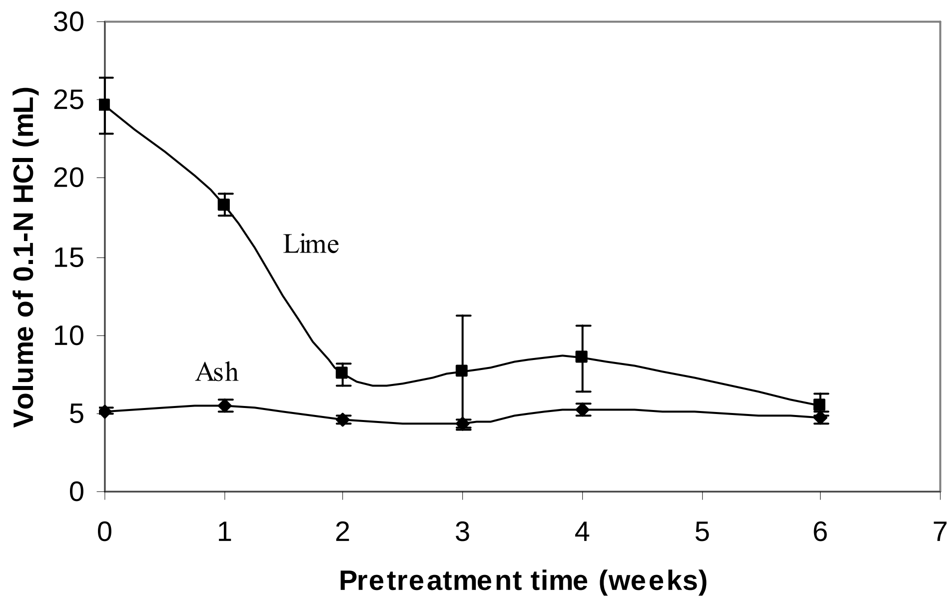


Figure 5-6. Titration of raw bagasse treated with lime and ash bagasse fermentation residue per 30 mL of pretreatment mixture.

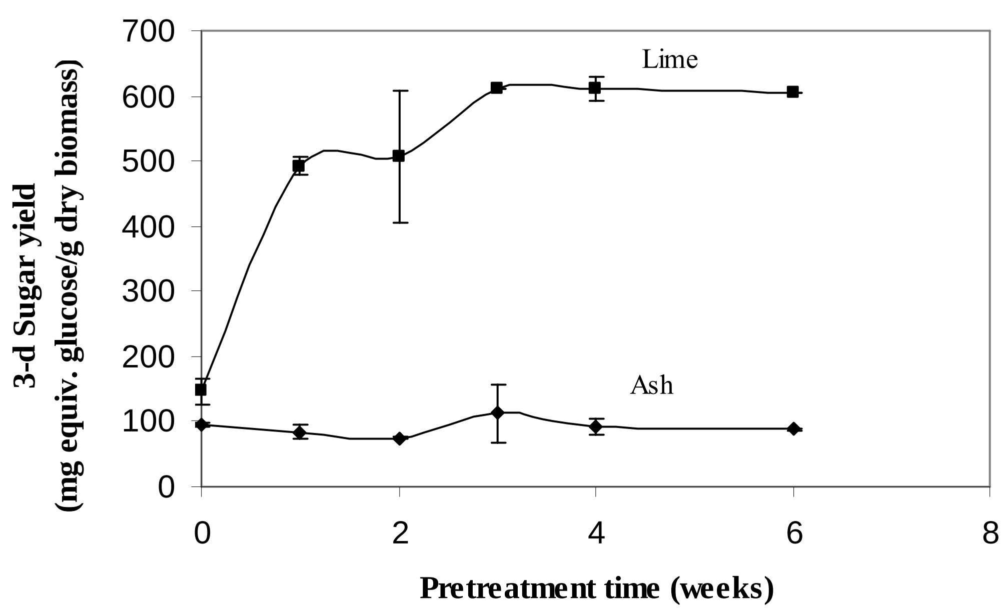


Figure 5-7. Sugar yield for lime- and ash-pretreated bagasse fermentation residue at 20 FPU/g biomass.

The sugar yield is shown in Figure 5-7. It shows that the glucose yield for lime-pretreated bagasse increased from 147 to 611 mg of glucose equivalent/g dry biomass. In contrast, ash-pretreated bagasse had almost no change at 95 mg of glucose equivalent/g dry biomass. The decrease in titrated acid equivalent correlated very well with glucose yield and therefore can be used as an indicator of the ability of a particular type of ash to pretreat biomass.

5.3 Conclusion

This study demonstrates that ash can be used to pretreat biomass. Ash from raw poplar wood ash was effective in pretreating poplar wood; however, ash from bagasse fermentation residues was not useful in pretreating bagasse. The raw poplar wood has appreciable mineral content that is alkaline when subjected to 550^{o}C temperatures. In contrast, residues from mixed-acid bagasse fermentations have no mineral content that is alkaline when subjected to 550^{o}C temperatures. Apparently, the acidic fermentation extracts alkaline minerals from the residues.

CHAPTER VI
LIGNIN FERMENTATION

6.1 Poplar Wood

The purpose of this experiment was to determine the fate of lignin in mixed-acid fermentation. This experiment requires a source of relatively pure lignin that is not chemically altered from native lignin. To meet this requirement, enzymatically liberated lignin was prepared from ball-milled poplar wood. Poplar wood was sieved through a 40-mesh sieve. Poplar wood (12 g) was added to 485 g of zirconium balls. The substrate was ball-milled for 3 d. Ball-milled poplar wood (25 g) was added to 500 mL of deionized water, 25 mL of citrate buffer, 15 mL of sodium azide, 5.5 mL of cellobiase, and 25 mL of cellulase enzyme (60 FPU/g biomass). Hydrolysis was performed at 50^{o}C for 3 d and the sugar concentration profile was measured using HPLC. Sugar concentrations monitored during the hydrolysis are shown in Figure 6-1.

The biomass was washed three times and hydrolyzed again using 500 mL of deionized water, 25 mL of citrate buffer, 15 mL of sodium azide, 5.5 mL of cellobiase, and 25 mL of cellulase enzyme. Sugar concentrations after the second hydrolysis are shown in Figure 6-2. During the first hydrolysis, the glucose and xylose concentrations increased to 7.8 g/L and 4.2 g/L, respectively. The glucose and xylose concentrations for the second hydrolysis were 1.17 g/L and 0.804 g/L, respectively (Figure 6-2). In both cases, hydrolysis was almost complete after 45 h. The total mass of sugars removed was 6.83 g in the first hydrolysis and 1.123 g in the second hydrolysis. The composition of the original poplar wood and the residue after the second hydrolysis is shown in Table 6-1.

Table 6-1. Composition of original poplar wood and residue

Components	Raw poplar wood	Lignin residue
Acid insoluble lignin (%)	30.7	51.1
Acid soluble lignin (%)	2.8	4.6
Ash (%)	6.3	10.4
Total sugars (%)	60.3	33.9
Total (%)	100	100

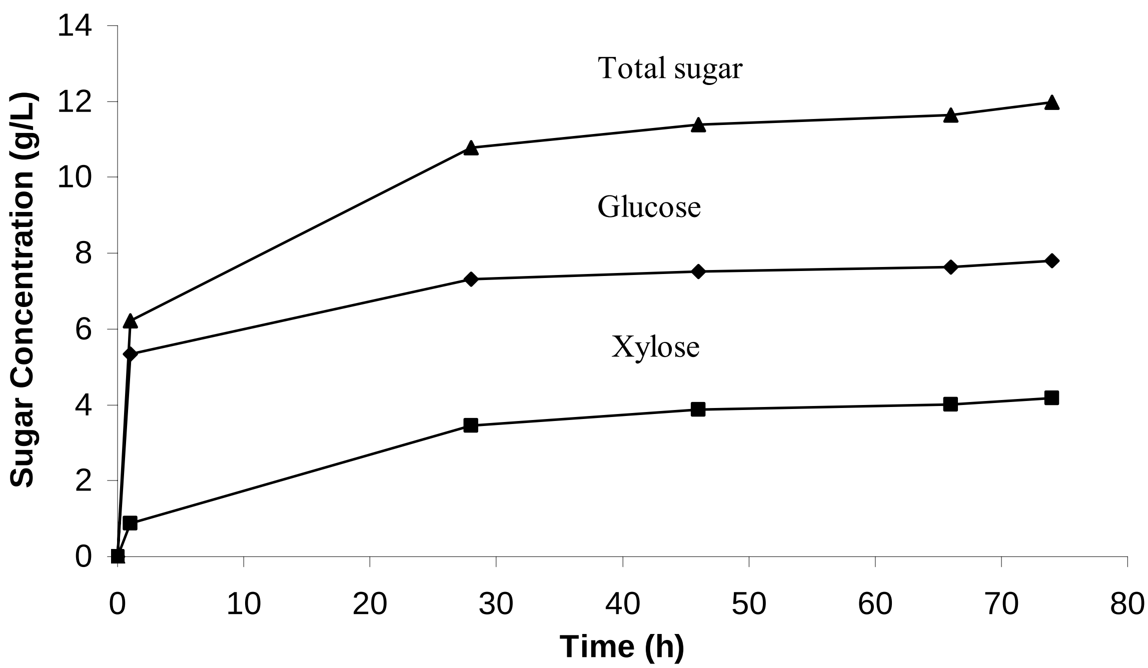


Figure 6-1. Sugar concentration in ball-milled poplar wood after the first hydrolysis.

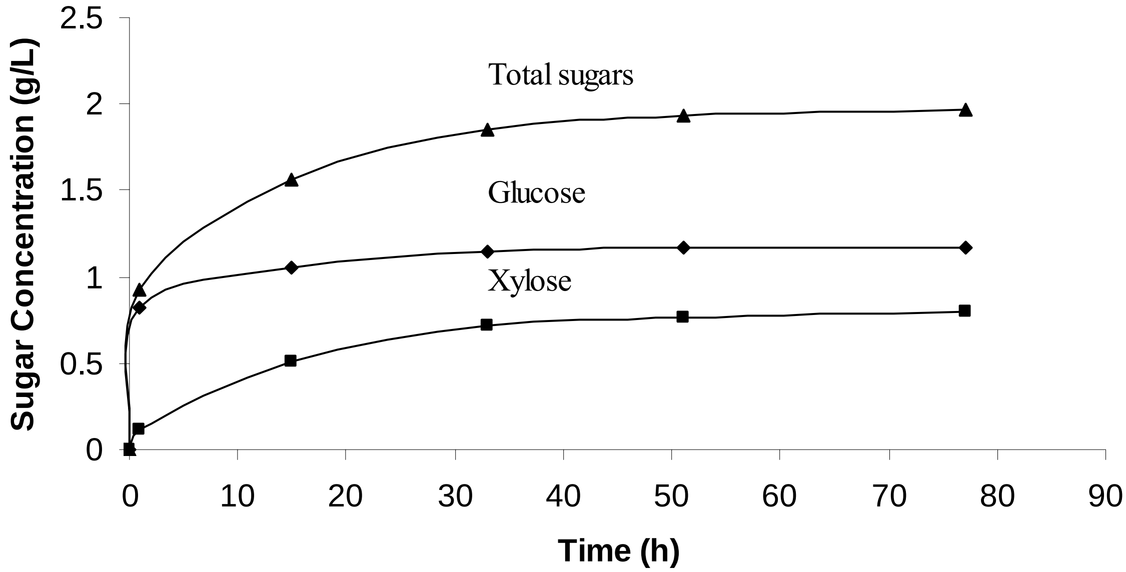


Figure 6-2. Sugar concentration in ball-milled poplar wood after the second hydrolysis.

The residue was washed with 6-M urea solution for 3 h to digest the residual enzymes from hydrolysis. The biomass was washed five times to remove residual urea. Solid samples were taken to analyze the residue (Table 6-1) and 1.4 g lime was added to the remaining 12 g of residue with 200 mL of distilled water. Air-lime pretreatment was performed on the residual lignin and hydrolytic lignin (Sigma Aldrich, Cat. No. 37,107-6) at 50°C for 6 weeks. There was no washing of the residue after the pretreatment so as to make solubilized lignin available to the microorganisms.

Fermentation was performed on the pretreated samples. The following experiments were performed:

- F1: 6 g of 1-h pretreated chicken manure
- F2: 6 g of 1-h pretreated chicken manure and 12 g of untreated lignaceous residue
- F3: 6 g of chicken manure and 12 g of air-lime pretreated lignaceous residue

- F4: 6 g of chicken manure and 12 g of air-lime pretreated hydrolytic lignin.

To each fermentor, 3 g of $CaCO_3$, 280 mL of anaerobic (deoxygenated) water, 0.1 g of urea, 0.2 g of inorganic nutrients and 200 μL of iodoform were added. Inoculum (40 mL) from the previous rice straw/chicken manure fermentation was used. The results from the fermentations are shown in Figure 6-3.

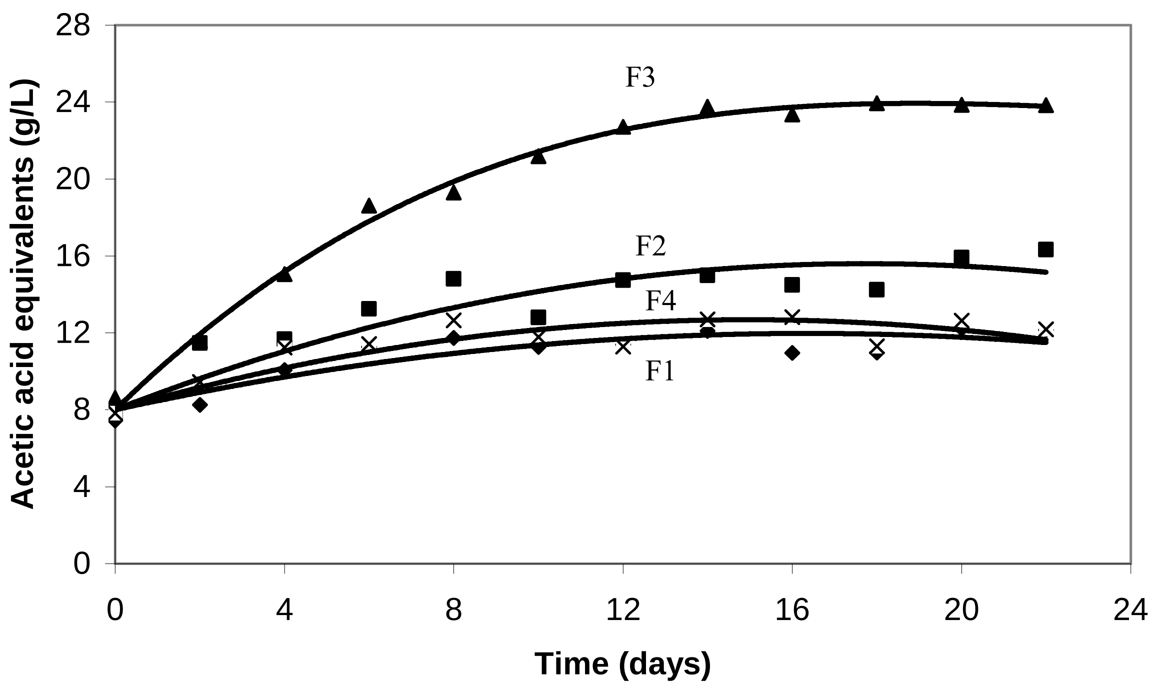


Figure 6-3. Acetic acid equivalent in lignaceous residue fermentation.

From Figure 6-3, the initial acid concentration was 8 g/L. After 22 d of fermentation, the final total acetic acid equivalent was 12 g/L in both pretreated chicken manure (F1) and air-lime pretreated hydrolytic lignin with chicken manure (F4). The fact that there is no difference in the acetic acid equivalents in the presence of air-lime pretreated commercial lignin (F4) indicates that hydrolytic lignin is not fermentable, even if it is pretreated. This might be due to the fact that the lignin is modified during its preparation as hydrolytic lignin.

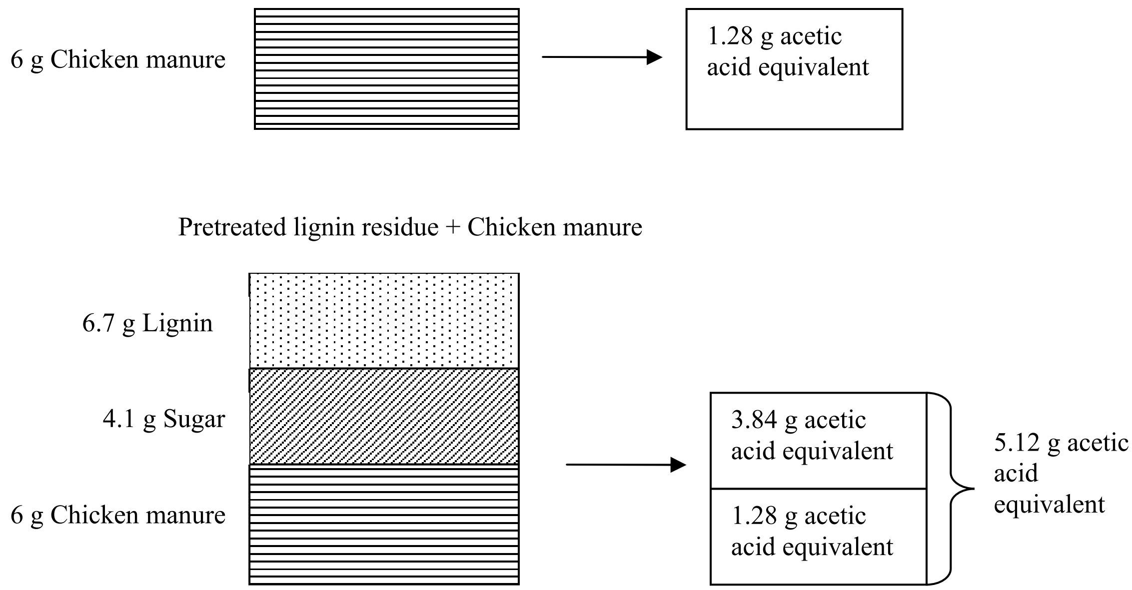


Figure 6-4. Acetic acid equivalent produced from biomass fermentation.

For untreated lignaceous residue (F2), the acetic acid equivalent increased from 8 to 16 g/L. The pretreated lignaceous residue (F3) increased from 8 to 24 g/L. The difference between F2 and F3 is due to the air-lime pretreatment. The clear difference between F2, F3, and F1 shows that the lignaceous residue was fermented by the marine microorganisms. The acetic acid equivalent produced from the biomass is shown in Figure 6-4. The acetic acid equivalent produced from chicken manure alone is 1.28 g. For the pretreated lignaceous residue (F3) and chicken manure, the acetic acid equivalent produced is 5.12 g (Figure 6-4). From pretreated lignaceous residue alone, the mass of acetic equivalents generated was 3.84 g and the mass of total acids was 2.37 g. The mass of sugars in the residue before fermentation was 4.07 g. The acid yield was 0.22 g of total acids/g of VS fed, which is lower yields for air-lime pretreated biomass (Tables 4-2 and 4-3). This experiment does not conclusively demonstrate that the lignin residue is fermentable from the mixed acids fermentation.

6.2 Bagasse

In this experiment, enzymatically liberated lignin was prepared from ball-milled bagasse. Bagasse was sieved through a 40-mesh sieve. Bagasse (12 g) was added to 485 g of zirconium balls. The substrate was ball-milled for 3 d. Ball-milled bagasse (25 g) was added to 500 mL of deionized water, 25 mL of citrate buffer, 15 mL of sodium azide, 5.5 mL of cellobiase, and 25 mL of cellulase enzyme (60 FPU/g biomass). Hydrolysis was performed at 50^{o}C for 3 d. The liquid hydrolyzate was discarded and the residue was washed. The biomass was ball-milled again for 3-d and hydrolyzed again using 500 mL of deionized water, 25 mL of citrate buffer, 15 mL of sodium azide, 5.5 mL of cellobiase, and 25 mL of cellulase enzyme at 50^{o}C for 3 d.

The hydrolyzate was discarded again and the residue was washed and ball-milled for the third time for 3 d. The residue was hydrolyzed again using 500 mL of deionized water, 25 mL of citrate buffer, 15 mL of sodium azide, 5.5 mL of cellobiase, and 25 mL of cellulase enzyme at 50^{o}C for 3 d. The residue was washed with 6-M urea solution for 3 h to digest the residual enzymes from hydrolysis. The biomass was washed five times to remove residual urea. The composition of the residue after the third hydrolysis is shown in Table 6-2. The biomass residue was prepared in triplicate and the mass of the residue before pretreatment was 7 g.

Table 6-2. Composition of original bagasse and residue

Components	Raw bagasse	Lignin residue
Acid insoluble lignin (%)	18.1	33.6
Acid soluble lignin (%)	1.2	1.8
Ash (%)	15	36
Glucose (%)	32.6	5.0
Xylose (%)	22.6	4.8
Total (%)	89.5	81.2

Lime (1.5 g) was added to the remaining 7 g of residue with 200 mL of distilled water. Air-lime pretreatment was performed on the residual lignin for 6 weeks at 50^{o}C. There was no washing of the residue after the pretreatment so as to make solubilized lignin available to the microorganisms.

Fermentation was performed by first determining the quantity of liquid left after pretreatment. Chicken manure (2 g), 2 g of $CaCO_3$, 0.1 g of urea, 0.2 g of inorganic nutrients and 200 μL of iodoform were added to six rotary fermentors. F1–F3 had pretreated liberated bagasse lignin and the glucose and xylose content of the bagasse lignin were compensated for in F4–F6 (Table 6-3). The glucose and xylan in F1–F3 were made available in the control experiments (F4–F6) in the form of α-cellulose and birchwood xylan respectively. Anaerobic media was added to F1–F3 to make a total volume of 260 mL. The amount of glucose and xylose in liberated bagasse lignin was compensated for by adding 0.35 g of α-cellulose and 0.35 g of birchwood xylan to the control F4–F6, and 260 mL of anaerobic water was added. Inoculum (40 mL) from previous rice straw/chicken manure fermentation was used in all the fermentors. The results from the fermentations are shown in Figure 6-5.

Table 6-3. Components in the fermentors

Components	F1–F3	F4–F6
Substrates	Enzymatically liberated bagasse lignin, chicken manure	α-cellulose, birchwood xylan, and chicken manure
Total liquid volume in each fermentor (L)	0.3	0.3
$CaCO_3$ (g)	2	2
Urea (g)	0.1	0.1
Inorganic nutrients (g)	0.2	0.2

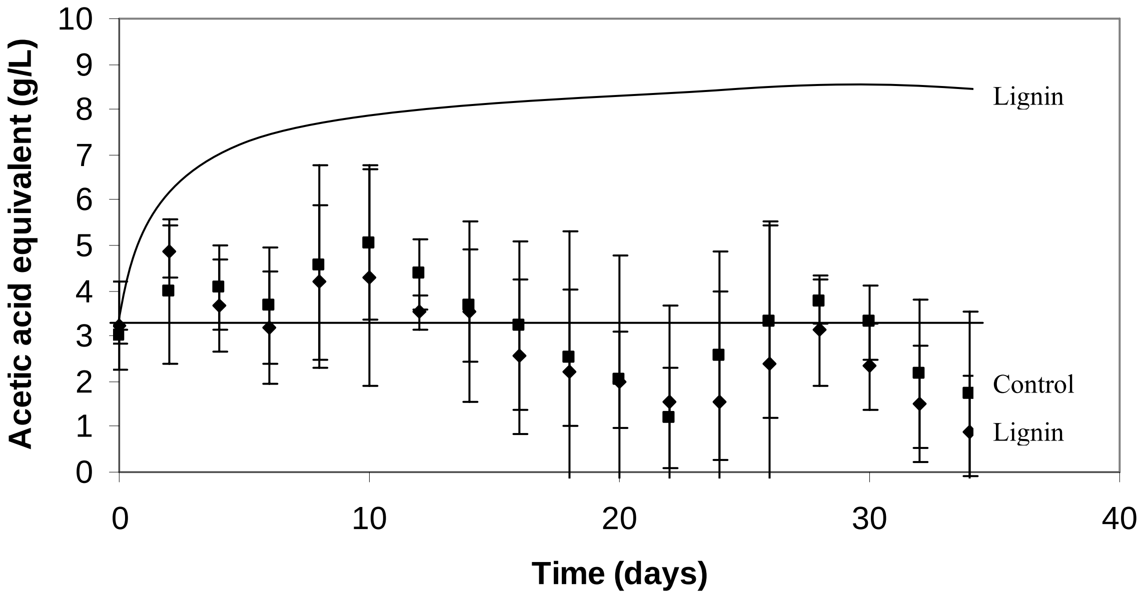


Figure 6-5. Total acids concentration of enzymatically liberated bagasse lignin (Error bars are ± 2σ).

From Figure 6-5, there is no difference between pretreated enzymatically liberated bagasse lignin and the control. The error bars (2 standard deviation) merge into each other, showing that there is no statistical difference between the lignin and the control at 95% confidence interval. The result does not show that solubilized lignin is fermented to organic acids. If this were the case, the total acid concentrations from the solubilized lignin would be significantly higher than the control. Using a typical yield for air-lime pretreated biomass, product concentrations from lignin fermentation should be about 9 g/L acetic acid equivalent if lignin were fermented.

6.3 Conclusion

Experiments on the fermentation of enzymatically liberated lignin from both poplar wood and bagasse do not show that solubilized lignin could be fermented to organic acids by using a mixed culture of marine microorganisms. So the question that arises is why are some of the conversions obtained using a mixed culture of marine microorganisms higher than the percentage of total sugars available? The answer lies in the fact that solubilized lignin is accounted for as a digested component in the mass balance (Figure 1-6). This is because any biomass component in the liquid or gas phase is considered digested. So it is true that conversions in mixed culture fermentations can be higher than the total sugars available but that does not mean solubilized lignin is fermented to organic acids.

CHAPTER VII
USING AMMONIUM BICARBONATE AS A BUFFER COMPARED TO CALCIUM CARBONATE

The objective of this set of experiments was to compare product concentrations of carboxylic acid fermentations using ammonium bicarbonate and calcium carbonate at mesophilic (40^{o}C) conditions. In this work, easily digestible biomass (waste office paper and raw chicken manure) was used to prevent the need for lime pretreatment; therefore there is no residual calcium from the pretreatment. Batch fermentations were performed and product concentrations were measured. Three experiments were performed as described below. The detailed experimental results are shown in Appendix N (Tables N7A–N7D).

7.1 Experiment 1

Comparing calcium carbonate and ammonium bicarbonate using inoculum from rice straw/chicken manure fermentations

Waste paper (16 g), 4 g of chicken manure, 0.2 g of urea, 0.3 g of inorganic nutrients, 240 mL of deoxygenated water and 10 mL of inoculum (from previous rice straw/chicken manure fermentations) were added to all three fermentors (F1–F3) initially. Every 2 days, iodoform (120 μL of 20 g /L in ethanol) was added to inhibit methanogens and 3 mL of liquid was taken as a sample. The fermentations were performed in a rotary fermentor (Figure 2-1).

- F1: 4 g of $CaCO_3$ was added initially and every 16 days
- F2: 1 g of NH_4HCO_3 was added initially and every 4 days thereafter
- F3: 4 g of NH_4HCO_3 was added initially and every 16 days

The total acid concentration and pH in Experiment 1 are shown in Figures 7-1 and 7-2.

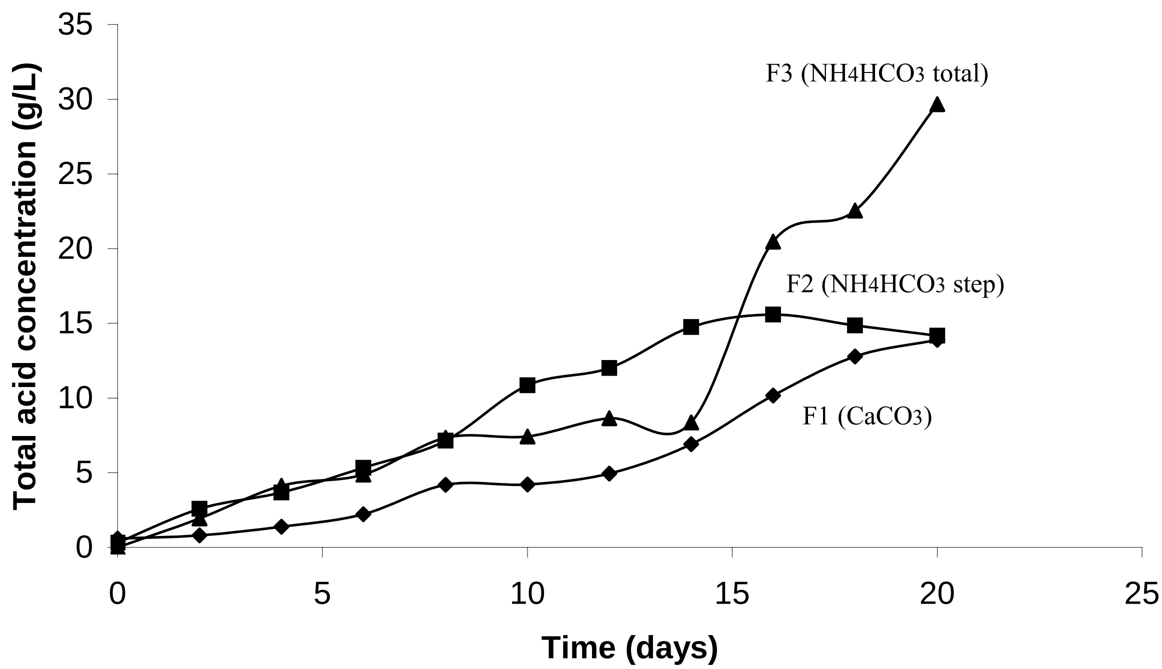


Figure 7-1. Total acid concentrations in Experiment 1.

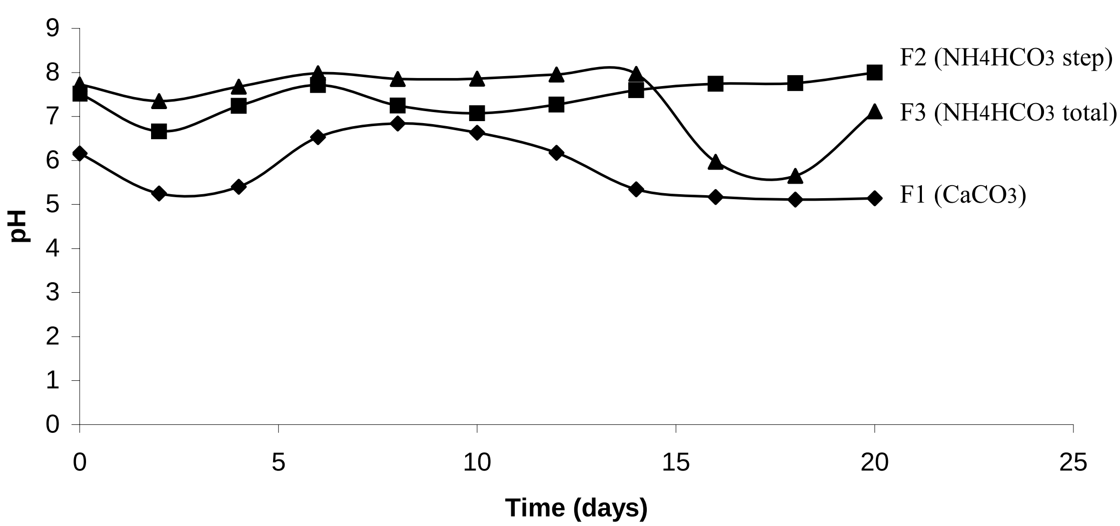


Figure 7-2. pH in Experiment 1.

From Figure 7-1, F3 with ammonium bicarbonate added in one batch had the highest acid concentration of 30 g/L compared to 14 g/L in F1 and F2. Initially, the pH in F3 was higher than F2, but on the 16th day of fermentation, the pH dropped from 8 to 6 resulting in an increase in total acid concentration from 8 g/L to 20 g/L. The percentage of acetate in the total acids is 60% in F1, 92% in F2 and 87% in F3. The percentage of acetate produced is higher in ammonium bicarbonate fermentations than calcium carbonate fermentations.

7.2 Experiment 2

Comparing calcium carbonate and ammonium bicarbonate using inoculum from Experiment 1

Two sets of experiment were performed using inoculum from Experiment 1:

a) Continuation of Experiment 1 with addition of fresh feed and deoxygenated water

b) Start of a new experiment with fresh feed and deoxygenated water using inoculum from Experiment 1.

The detailed descriptions for both experiments are given below:

a) Inoculum (50 mL) was taken from each fermentor for Experiment 2b. Waste paper (16 g), 4 g of chicken manure, 0.2 g of urea, 0.3 g of inorganic nutrients, 50 mL of deoxygenated water were added to all three fermentors (F1–F3). Iodoform (120 μL of 20 g/L in ethanol) was added every 2 days to inhibit methanogens and 3 mL of liquid was taken as a sample. The following fermentations were employed:

- F1: 6 g of $CaCO_3$ was added initially and every 16 days
- F2: 2 g of NH_4HCO_3 was added initially and every 4 days thereafter
- F3: 6 g of NH_4HCO_3 was added initially and every 16 days

b) Waste paper (16 g), 4 g of chicken manure, 0.2 g of urea, 0.3 g of inorganic nutrients, 220 mL of anaerobic water and 30 mL of inoculum (Experiment 1) were added to all three fermentors (F1–F3). Iodoform (120 μL of 20 g/L in ethanol) was added every 2 days to inhibit methanogens and 3 mL of liquid was taken as a sample. The following fermentations were employed:

- F1: 6 g of $CaCO_3$ was added initially and every 16 days
- F2: 2 g of NH_4HCO_3 was added initially and every 4 days thereafter
- F3: 6 g of NH_4HCO_3 was added initially and every 16 days

Experiment 2a

The total acid concentration and pH in Experiment 2a are shown in Figures 7-3 and 7-4, respectively. From Figure 7-3, F3 with ammonium bicarbonate added all at once had the highest acid concentration of 39 g/L compared to 32 g/L in F1 and 23 g/L in F2. The pH in F1 was about 5.5, 8 in F2 and 7.5 in F3. The high pH in F2 contributed to the low product concentrations compared to F1 and F3.

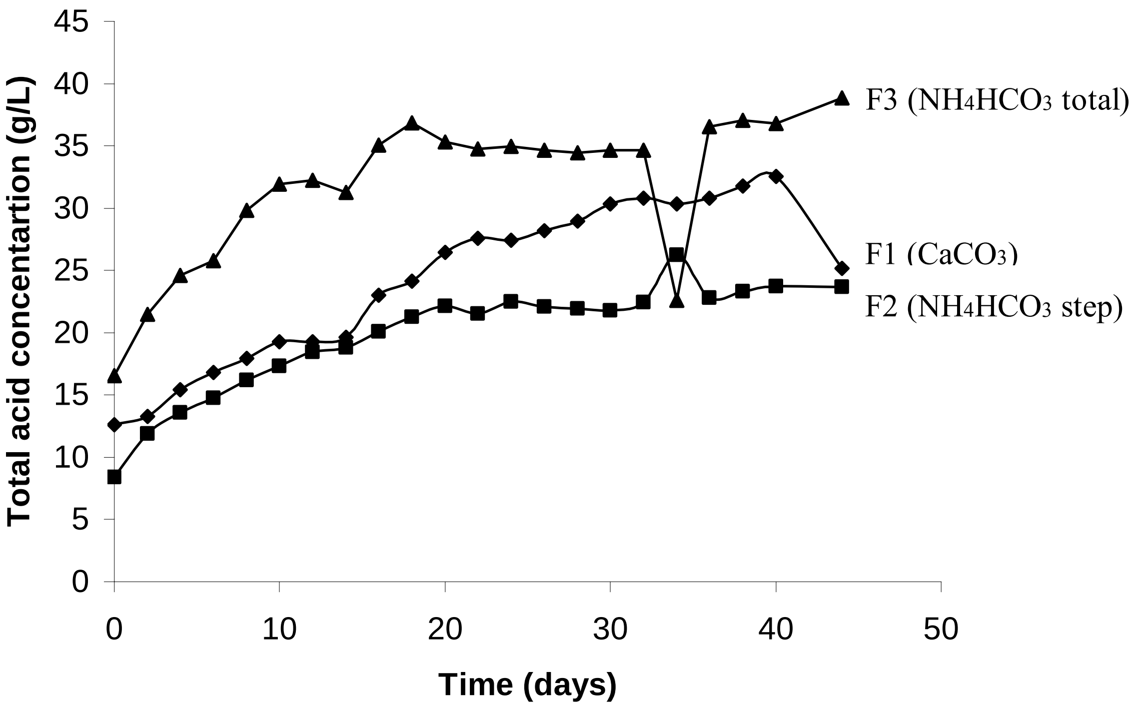


Figure 7-3. The total acid concentrations in Experiment 2a.

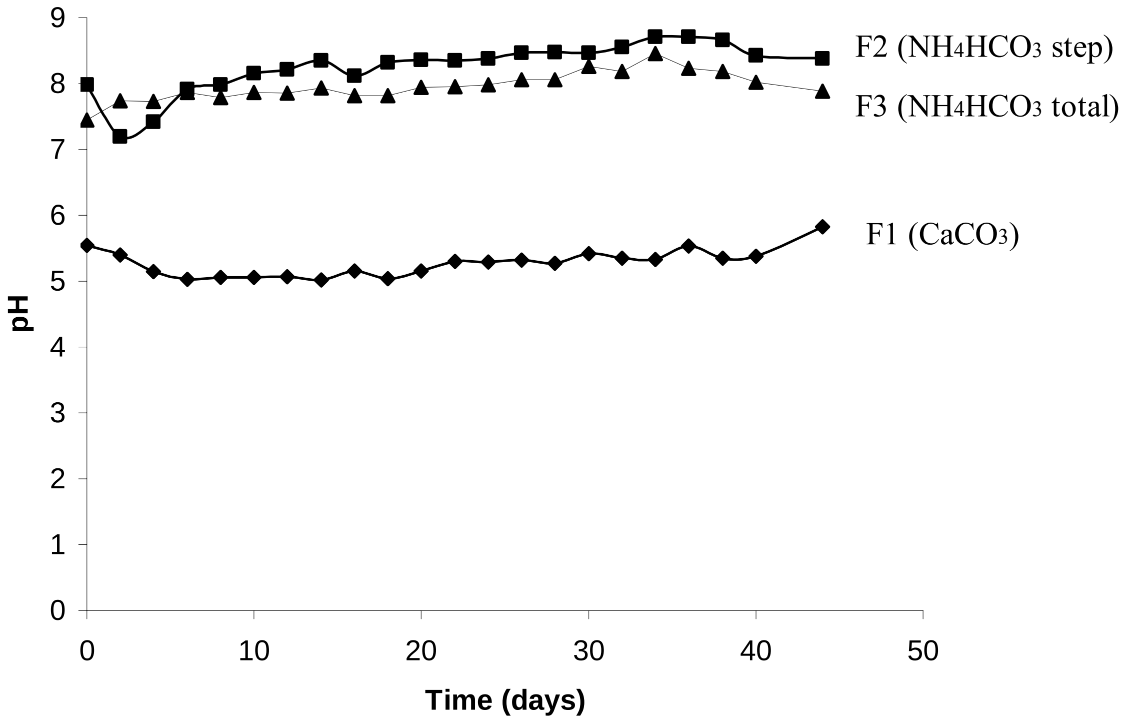


Figure 7-4. pH in Experiment 2a.

Experiment 2b

The total acid concentration and pH in Experiment 2b are shown in Figures 7-5 and 7-6 below. From Figure 7-5, F1 with calcium carbonate had the highest acid concentration of 13 g/L compared to 9 g/L in F2 and F3. The pH in F1 was about 6, 8 in F2 and 8 in F3. The high pH in F2 and F3 contributed to the low product concentrations compared to F1.

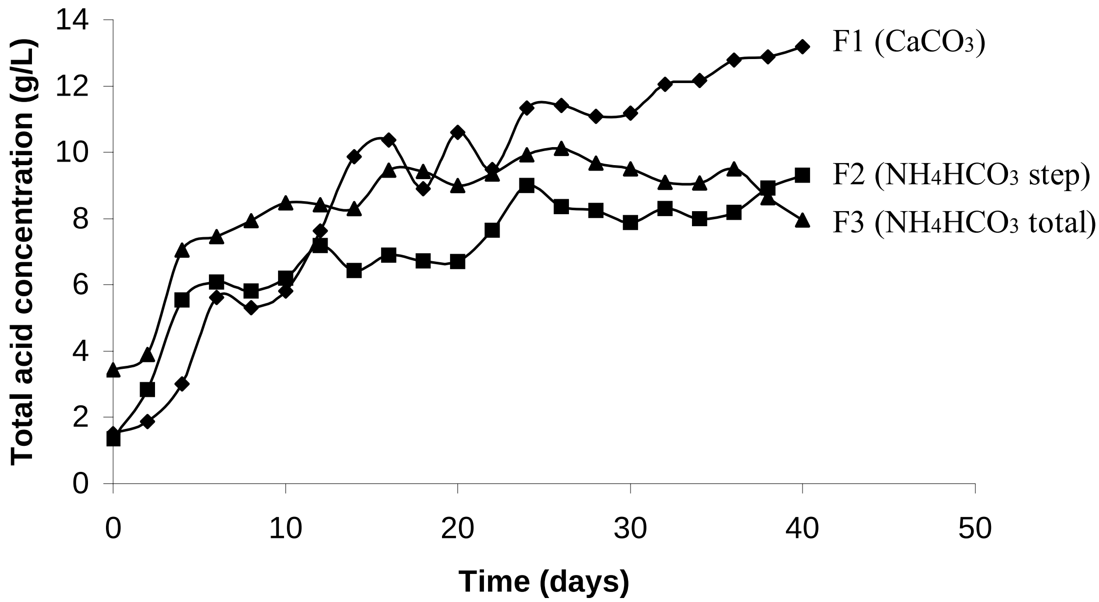


Figure 7-5. The total acid concentrations in Experiment 2b.

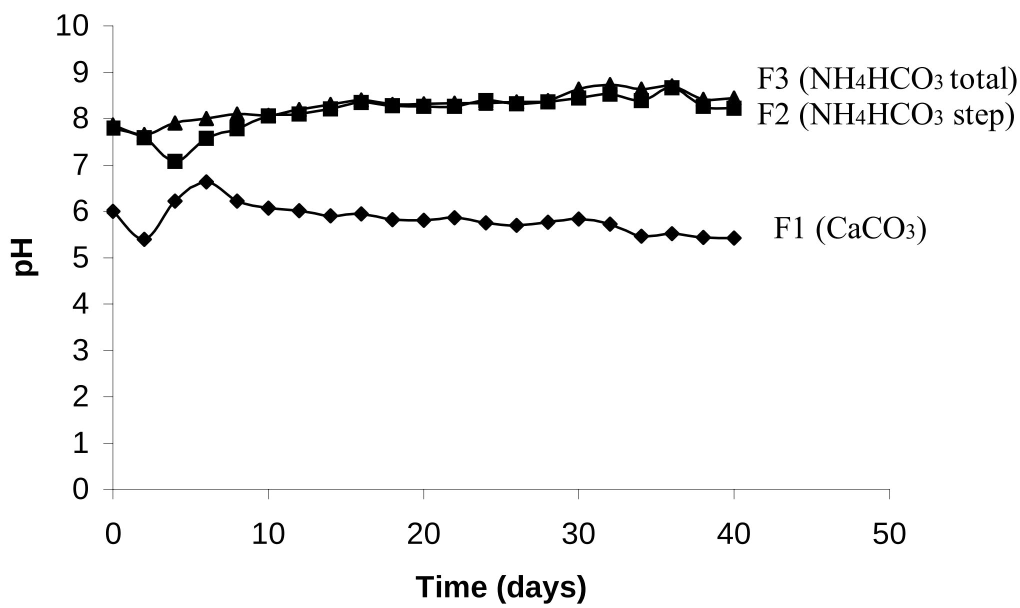


Figure 7-6. pH in Experiment 2b.

7.3 Experiment 3

Effect of pH

The objective of this experiment was to determine if pH or the presence of ammonia was responsible for the high product concentrations in Experiments 1 and 2a.

Waste paper (16 g), 4 g of chicken manure, 0.2 g of urea, 0.3 g of inorganic nutrients, 240 mL of deoxygenated water and 10 mL of inocula (Experiment 2) were added to all three fermentors (F1–F3) initially. Iodoform (120 µL of 20 g/L in ethanol) was added every 2 days to inhibit methanogens and 3 mL of liquid was taken as a sample. The following fermentations were performed:

- F1: 4 g of $CaCO_3$ was added and lime was added to adjust pH to 7
- F2: NH_4HCO_3 was added to adjust pH to 7 using inoculum from F2 (Experiment 2 a)
- F3: NH_4HCO_3 was added to adjust pH to 7 using inoculum from F3 (Experiment 2 a)

After 16 days of fermentation, fresh feed (16 g waste paper, 4 g chicken manure) as well as $CaCO_3$ and ammonium bicarbonate dosages listed above were reintroduced into the fermentors. The total acid concentrations and pH are listed in the results. If the pH was already 7 or very close to it, no adjustment was done.

Total acid concentrations and pH for Experiment 3 are shown in Figures 7-7 and 7-8, respectively. From Figure 7-7, F2 and F3 with ammonium bicarbonate had the highest acid concentrations of 30 g/L compared to 15 g/L in F1. Although the pH was adjusted to 7 every 2 days, the pH before adjustment in F1 was about 6.5, 6.8 in F2, and 7 in F3. From Figure 7-8, the ammonium bicarbonate buffer is more resistant to changes in pH compared with calcium carbonate/lime system. This experiment demonstrates that the higher pH in Experiment 2 was responsible for the low product concentration.

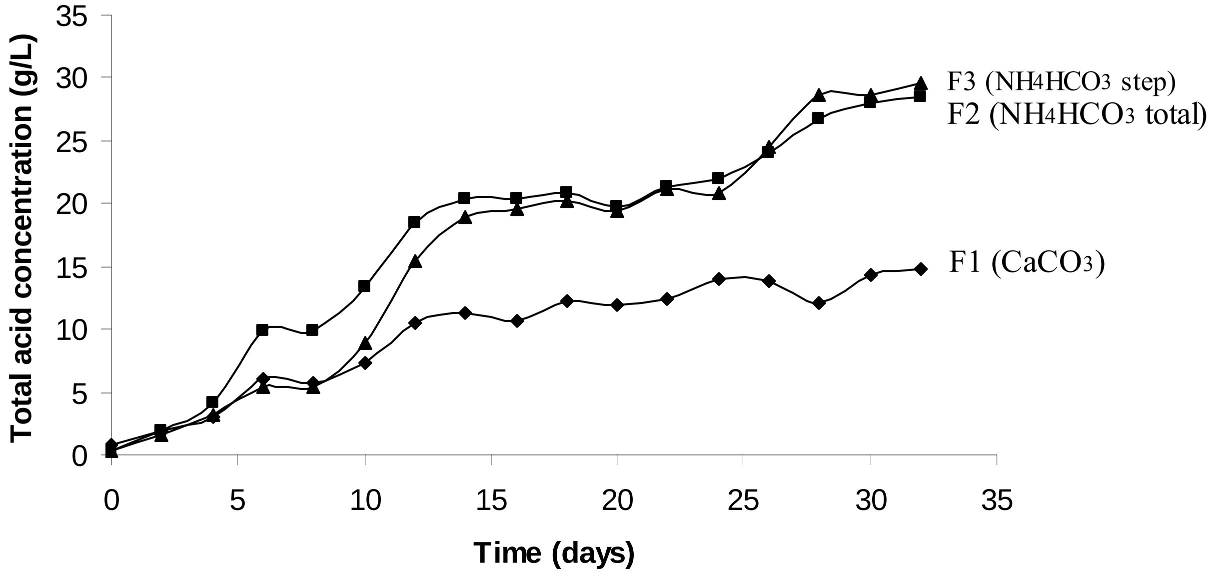


Figure 7-7. The total acid concentrations in Experiment 3.

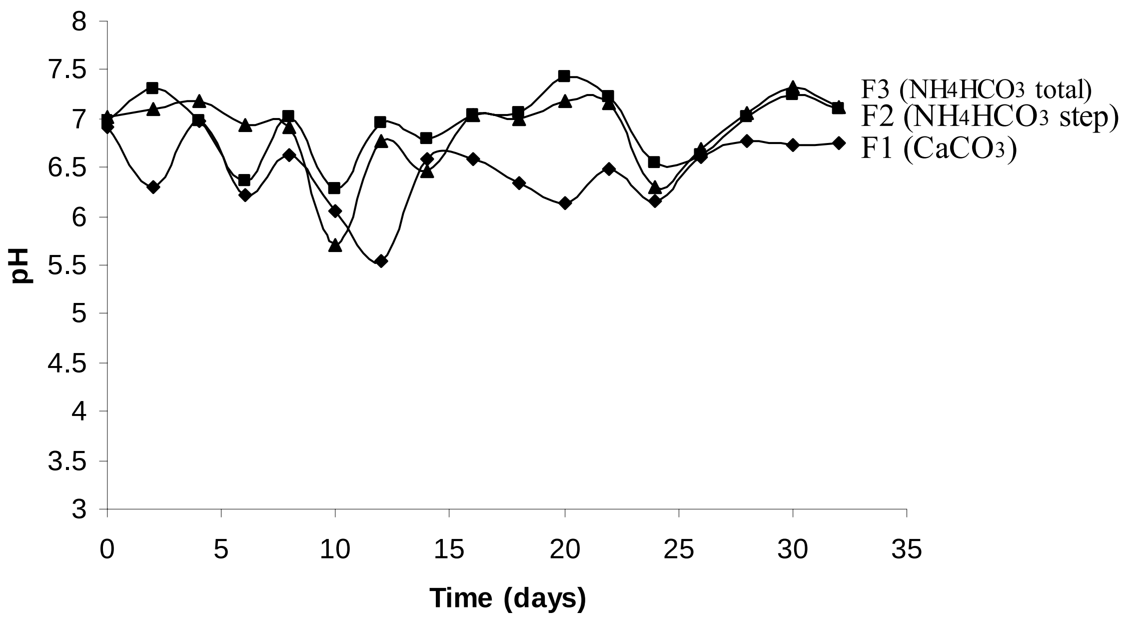


Figure 7-8. pH in Experiment 3.

7.4 Conclusion

It has been demonstrated that using ammonium bicarbonate produces a higher acid concentration at mesophilic conditions. The following conclusions have been made:

1) The total product concentration using ammonium bicarbonate is almost double the product concentration using calcium carbonate if the pH of ammonium bicarbonate fermentation is around 7.
2) The acetate content of total acids using ammonium bicarbonate is about 90% at mesophilic fermentation. This is higher than 60% for mesophilic fermentations using calcium carbonate.
3) The ammonium bicarbonate fermentation is pH sensitive and if the pH is 8 or above, it is possible product concentration will not be high. It has been demonstrated that if the pH is controlled at 7, the product concentration from ammonium bicarbonate was still double the value for calcium carbonate fermentation controlled with lime at the same pH. The buffer system for ammonium bicarbonate was more resistant to changes in pH compared to the lime/calcium carbonate system.

CHAPTER VIII
CONCLUSIONS AND RECOMMENDATIONS

In the countercurrent fermentation of rice straw and chicken manure, the highest acid productivity of 1.69 g/(L·d) occurred at a concentration of 32.4 g/L in Fermentation Train E (LRT = 19.2 d and VSLR = 5.87 g/(L·d)). This fermentation train (Train E) had the shortest LRT; Fermentation Train F (LRT = 26.3 d and VSLR = 8.06 g/(L·d)) had the longest LRT. Fermentation Train B (LRT = 23.7 d and VSLR = 3.45 g/(L·d)) with a concentration of 25 g/L had the highest conversion (0.692 g VS digested/g VS fed) and yield (0.29 g total acids/g VS fed). Fermentation Train B had the highest conversion and yield because it had the lowest VSLR, which made more complete use of the biomass. The highest selectivity of 0.57 g total acids/g VS digested was in Fermentation Train C (LRT = 24.0 d and VSLR = 6.47 g/(L·d)).

The acid productivity increased as VSLR increased. There is a decrease in conversion (x), and yield (y) as VSLR increased. The selectivity (s) was essentially constant as VSLR increased. At a high VSLR, the microorganisms digested more biomass leading to a high acid productivity; however, yield, and conversion were lower because only a small fraction of the total biomass fed was digested. On the other hand, at low VSLR, the microorganisms were limited by the amount of digestible biomass available. Therefore, the acid productivity was low but the yield, and conversion were higher because the microorganisms consumed both the reactive and recalcitrant biomass components.

The average error between the experimental and predicted total acid concentrations was 6.41%, and the average error between the experimental and predicted conversion was 6.55%. The highest error in total acid total carboxylic acids was 16.2% and the highest error in conversion was 22.1%. The CPDM “map” for the 80% rice straw/20% chicken manure fermentation system in an industrial fermentor (300 g VS/L of liquid) shows that more than 90% conversion could be obtained at a LRT of 10 d and VSLR of 4 g/(L·d). The “map” predicts a total acid concentration of 60 g/L at LRT of 30 d, VSLR of 10 g/(L·d) and a conversion of 50%. At a VSLR of 4 g/(L·d) and LRT of 30 d, a total acid concentration of 50 g/L could be obtained at 80% conversion.

In the fixed-bed fermentation system, the maximum total acid concentration for F1 in Train A was 34.2 g/L and the maximum acid concentration in F2–F4 was ~44 g/L. The maximum total acid concentration in F1 in Train B was 30.5 g/L and the maximum acid concentration in F2–F4 was ~48 g/L. Although there was almost no difference in the total acid concentrations of F2–F4 in both sets, the times at which the maximum peak occurred increased from F2 to F4. The conversion in each of the fermentors in Train A varied from 0.821–0.879 g VS digested/g VS fed and the yield ranged from 0.489–0.609 g total acids/g VS fed. The conversion and yield in Train B were 0.741–0.914 g VS digested/g VS fed and 0.563–0.669 g total acids/g VS fed.

The maximum total acid concentration for F1 in Train C was 25.7 g/L and the maximum acid concentration in F2–F4 was ~34 g/L. The maximum total acid concentration in F1 in Train D was 21.8 g/L and the maximum acid concentration in F2–F4 was ~31 g/L. Although there was almost no difference in the total acid concentrations of F2–F4 in both sets, the times at which the maximum peak occurred generally increased from F2 to F4. The conversion in each of the reactors in Train C varied from 0.431–0.820 g VS digested/g VS fed and the yield ranged from 0.255–0.689 g total acids/g VS fed. The conversion and yield in Train D were 0.547–0.969 g VS digested/g VS fed and 0.315–0.808 g total acids/g VS fed.

The results indicate that fermentation data from one fermentor could be used to obtain the product concentrations in other fermentors; however, there was always a lot of scatter. The major cause for the scatter likely stems from the fact that the fermentors are not mixed. There is the possibility of channeling and local concentration differences within the fermentor which could account for the scatter. The predictability of the model can be improved by modifying the fermentation data collection process. This will involve preventing channeling, getting uniform product distribution within the fermentors, and preventing leakage.

CPDM was extended to model the fixed-bed fermentation system. The correlation coefficient for the four fermentors in Train A was between 0.71–0.84. The same equation was used on to obtain the product concentrations in Train B. The correlation coefficient for the four fermentors in Train B was 0.67–0.80. The predictability of Train A was comparable to results in Train B.

CPDM was extended to the round robin system to predict steady total acid concentrations. The noise in the predicted total acid concentrations was higher at lower liquid transfer rates compared to high liquid transfer rates. In the round robin system, an increase in the time it takes to do a transfer increases the total acid concentration. This is because the longer it takes to do a transfer, the more the acids build up and the higher the total acid concentration.

It has been demonstrated in this study that ash can be used to pretreat biomass. Ash from raw poplar wood ash was effective in pretreating poplar wood; however, ash from bagasse fermentation residues was not useful in pretreating bagasse. The raw poplar wood has appreciable mineral content that is alkaline when subjected to 550^{o}C temperatures. In contrast, residues from mixed-acid bagasse fermentations had no mineral content that was alkaline when subjected to 550^{o}C temperatures. Apparently, the acidic fermentation extracted alkaline minerals from the residues.

Experiments on the fermentation of enzymatically liberated lignin from both poplar wood and bagasse do not show that solubilized lignin could be fermented to organic acids by using a mixed culture of marine microorganisms. Any solid biomass component that is in the liquid or gas phase is considered digested. So it is true conversions in mixed culture fermentations can be higher than the total sugars available, but that does not mean solubilized lignin was fermented to organic acids.

The total product concentrations using ammonium bicarbonate is almost double the product concentrations using calcium carbonate if the pH of ammonium bicarbonate fermentation is around 7. The acetate content of total acids using ammonium bicarbonate is about 90% at mesophilic fermentation. This is higher than 60% for mesophilic fermentations using calcium carbonate. The ammonium bicarbonate fermentation is pH sensitive and if the pH is 8 or above, it is possible product concentrations will not be high. It has been demonstrated that if the pH is controlled to 7, product concentrations from ammonium bicarbonate was still double the value for calcium carbonate fermentation controlled with lime at the same pH. The buffer system for ammonium bicarbonate was more resistant to changes in pH compared to lime/calcium carbonate system.

REFERENCES

Aiello-Mazzarri, C., 2002. Conversion of municipal solid waste to carboxylic acids by anaerobic countercurrent fermentation. PhD dissertation, Texas A&M University, College Station, Texas.

Anderson, R.C., Callaway, T.R., Van Kessel, J.A.S., Jung, Y.S., Edrington, T.S., Nisbet, D.J., 2003. Effect of select nitrocompounds on ruminal fermentation; an initial look at their potential to reduce economic and environmental costs associated with ruminal methanogenesis. Bioresource Technology 90(1): 59-63.

Anderson, R.C., Carstens, G.E., Miller, R.K., Callaway, T.R., Schultz, C.L., Edrington, T.S., Harvey, R.B., Nisbet, D.J., 2004. Effect of nitroethane administration on ruminal VFA production and specific activity of methane production. Journal of Animal and Feed Sciences 13: 23-26.

Bauchop, T., 1967. Inhibition of rumen microorganisms by methane analogues. J. Bacteriol. 94, 171-175.

Brune, A., Miambi, E., Breznak, J. A., 1995. Roles of oxygen and the intestinal microflora in the metabolism of lignin-derived phenylpropanoids and other monoaromatic compounds by termites. Appl. Environ. Microbiol. 61:2688-2695.

California Rice Commission’s Library on Rice Straw Utilization, www.westbioenergy.org/ricestraw/one.html (Accessed June, 2002)

Chadha, B.S., Kanwar, S.S., Saini, H.S., Garcha, H.S., 1995. Hybrid process for ethanol production from rice straw. Acta Microbiologica et Immunologica Hungarica, 42(1), 53-59.

Chan, W.N., Holtzapple, M.T. (2003). Conversion of municipal solid wastes to carboxylic acids by thermophilic fermentation. Appl. Biochem. Biotechnol. 111, 93-112.

Chang, V.S., Burr, B., Holtzapple, M.T., 1997. Lime pretreatment of switchgrass. Applied Biochemistry and Biotechnology 63, 3-19.

Chang, V.S., Nagwani, M., Holtzapple, M.T., 1998. Lime pretreatment of crop residues bagasse and wheat straw. Applied Biochemistry and Biotechnology 74, 135-159.

Chang V.S., Holtzapple, M.T., 2000. Fundamental factors affecting biomass enzymatic reactivity, Applied Biochemistry and Biotechnology 84-86, 1-37.

Chang, V.S., Nagwani, M., Kim, C.H., Holtzapple, M.T., 2001. Oxidative lime pretreatment of high-lignin biomass. Applied Biochemistry and Biotechnology 94, 1-28.

Chidthaisong, A., Watanabe, I., 1997. Methane formation and emission from flooded rice soil incorporated with ^{13}C-labelled rice straw. Soil Biol. Biochem. 29, 1173-1181.

Crawford, D. L., Pometto III, A. L., Crawford, R. L., 1983. Lignin degradation by *Streptomyces viridosporus*: isolation and characterization of a new polymeric lignin degradation intermediate. Appl. Environ. Microbiol. 45:898-904.

Datta, R., 1981. Acidogenic fermentation of corn stover. Biotechnology and Bioengineering 23, 61-77.

Fan, L. T., Lee, Y.-H., Gharpuray, M. M., 1982. The nature of lignocellulosic and their pretreatment for enzymatic hydrolysis. Adv. Bich. Eng. 23.157-187.

Fan, L. T., Lee, Y.-H., Gharpuray, M. M., 1987. Nature of cellulose material and enzymatic hydrolysis. In Cellulose Hydrolysis. 1-69.

Fukuzaki, S., Nishio, M., Nagai, S., 1990. Kinetics of the methanogenic fermentation of acetate. Applied Environmental Microbiology. 56. 3158-3163.

Hisashi, M., Danner, H., Neureiter, M., Thomasser, C., Braun, R., 2003. Effect of wood ash treatment on improving the fermentability of wood hydrolyzate. Biotechnology and Bioengineering 84(3), 390-394.

Holtzapple, M. T., 1993. Cellulose, hemicellulose, lignin, in: Macrae, R., Robinson, R. K., and Sadler, M. J. (Eds.), Encyclopedia of Food Science, Food Technology and Nutrition, vol. 2, pp. 758-767, pp. 2324-2333, pp. 2731-2738.

Holtzapple, M.T., Davison, R.R., Ross, M.K., Aldrett-Lee, S., Nagwani, M., Lee, C.M., Andelson, S.K., Kaar, W., Gaskin, D., Shirage, H., Chang, N.S., Chang, V.S., Loescher, M.E., 1999. Biomass conversion to mixed alcohol fuels using the MixAlco process. Applied Biochemistry and Biotechnology 77-79, 609-631.

Hutjens, M. F., Feed additives in dairy nutrition and industry and farm fermentation, University of Illinois, Urbana, Illinois. http://www.traill.uiuc.edu/uploads/dairynet/papers/Northeast%20Nut%20Conf%20Feed%20Additives.doc2.pdf. (Date accessed May, 2005)

Jeffries, T. W., 1990. Biodegradation of lignin-carbohydrate complexes. Biodegradation 1:163-176.

Kaar, W.E., Holtzapple, M.T., 2000. Using lime pretreatment to facilitate the enzymatic hydrolysis of corn stover. Biomass and Bioenergy 18, 189-199.

Kayhanian, M., 1999. Ammonia inhibition of high-solids biogasification: An overview and practical solutions. Environmental Technology 20, 355-365.

Kheshgi, H.S., Prince, R.C., Marland, G., 2000. The potential of biomass fuels in the context of global climate change: Focus on transportation fuels. Annual Reviews Energy & Environment 25: 199-244.

Kim, S., 2004. Lime pretreatment and enzymatic hydrolysis of Corn Stover. PhD dissertation, Texas A&M University, College Station, Texas.

Kunii, D., Levenspiel, O., 1969. Fluidization Engineering, Wiley, New York.

Lai, Y.Z., 2001. Chapter 10 Chemical degradation, in: Hon, D. N.-S., and Shiraisha, N. (Eds.), Wood and Cellulose Chemistry. Marcel Dekker, Inc., New York, Basel, pp. 443-512.

Latham M. J., Wolin M. J., 1977. Fermentation of cellulose by *Ruminococcus flavefaciens* in the presence and absence of *Methanobacterium ruminantium*. Appl. Environ. Microbiol. 34:297-301.

Li, X., 2002. Production of acetic acid from synthesis gas with mixed acetogenic microorganisms. PhD dissertation, Texas A&M University, College Station, Texas.

Loescher, M.E., 1996. Volatile fatty acid fermentation of biomass and kinetic modeling using the CPDM method. PhD dissertation, Texas A&M University, College Station, Texas.

Lynd, L.R., Cushman, J.H., Nichols, R.J., Wyman, C.E., 1991. Fuel ethanol from cellulosic biomass. Science 251, 1318-1323.

Lynd, L.R., Wyman, C.E., Gerngross, T. U., 1999. Biocommodity engineering. Biotechnology Progress 15, 777-793.

Lynd, L.R., Weimer, P.L., van Zyl, W.H., Pretorius, I.S., 2002. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiology and Molecular Biology Reviews 66(3), 506-577.

Martin, S. A., Macy, J. M., 1985. Effects of monensin, pyromellitic diimide and 2-bromoethanesulfonic acid on rumen fermentation in vitro. J. Anim. Sci. 60:544-550.

McCarty, A. J., 1987. Lignocellulose-degrading actinomycetes. FEMS Microbio. Rev. 46:145-163.

Mosier, N, Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2004. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology 96, 673-686.

Oba, M., Allen, M. S., 2002. Hypophagic effects of ammonium are greater when infused with propionate compared with acetate in lactating dairy cows, Nutrient Metabolism 1100-1104.

Otterby, D. E., Johnson, D. G., Cook, R. M., Towns, R., Erdman, R. A., Van Horn, H. H., Rogers, J. A., Clark, W. A., 1990. Dose response of dairy cows to ammonium salts of volatile fatty acids, J. Dairy Sci. 73(8), 2168-2178.

Parkin, G. F., Speece, R. E., 1980. Modeling toxicity in methane formation systems, ASCE National Conference on Environmental Engineering, New York.

Peters, J. P., Leedle, J.A.Z., Paulissen, J.B., 1989. Factors affecting the in vitro production of volatile fatty acids by mixed bacterial populations from the bovine rumen. J. Anim. Sci. 67: 1593-1602.

Prins, R. A., 1977. Biochemical activities of gut microorganisms, in: Clarke, R. T. J. and Bauchop, T. (Eds.), Microbial Ecology of the Gut. New York. Academic Press. pp. 73-184.

Ramachandra, M. D., Crawford, D. L., Hertel, G., 1988. Characterization of an extracellular lignin peroxidase of the lignocellulolytic actinomytes *Streptomyces viridosporus*. Appl. Environ. Microbiol. 54:3057-3063.

Ross, M. K., 1998. Production of acetic acid from waste biomass. PhD dissertation, Texas A&M University, College Station, Texas.

Ross, M. K., Holtzapple M. T., 2001. Laboratory method for high-solids countercurrent fermentations. Applied Biochemistry and Biotechnology, 94, 111-126.

Russell, J. B., Dombrowski, D. B., 1980. Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. Applied and Environmental Microbiology 604-610.

Russell, J. B., Martin, S. A., 1984. Effects of various methane inhibitors on the fermentation of amino acids by mixed rumen microorganisms. J. Animal Sci. 59:1329-1338.

Russell, J. B., Wilson, D. B., 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? J. Dairy Sci. 79:1503-1509.

Sauer, F. D., Teather, R. M., 1987. Changes in oxidation reduction potentials and volatile fatty acid production by rumen bacteria when methane synthesis is inhibited. J. Dairy Sci. 70:1835-1840.

South, C.R., Lynd, L.R., 1994. Analysis of conversion of particulate biomass to ethanol in continuous solids retaining and cascade bioreactors. Applied Biochemistry and Biotechnology 45/46, 467-481.

Thanakoses, P., 2002. Conversion of bagasse and corn stover to mixed carboxylic acids using a mixed culture of mesophilic microorganisms. PhD dissertation, Texas A&M University, College Station, Texas.

Thanakoses, P., Mostafa, N.A.A., Holtzapple, M.T., 2003. Conversion of sugarcane bagasse to carboxylic acids using a mixed culture of mesophilic microorganisms. Applied Biochemistry and Biotechnology. 105, 523-546.

Zeikus, J. G., 1980. Chemical and fuel production by anaerobic bacteria. Annual Rev. Microbiol. 34:423-464.

Zhang, R., Zhang, Z., 1999. Biogasification of rice straw with an anaerobic-phased solids digester system. Bioresource Technology, 68, 235-245.

Zhang, Y-H, P, Lynd, L. R., 2004. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplex cellulase systems. Biotechnology and Bioengineering. 88 (7), 797-824.

APPENDIX A
SHORT-TIME LIME TREATMENT OF RICE STRAW AND CHICKEN MANURE

Biomass was treated with calcium hydroxide (lime) in the presence of water and boiled for 1 h. The ground biomass and calcium hydroxide (0.1 g/g dry biomass) were placed in the tray and thoroughly mixed. Distilled water (enough to cover the material) was added to the dry mixture. The tray was then covered with aluminum foil and allowed to reach boiling temperature. The material was allowed to boil for 1 h. After boiling, the pretreated biomass is neutralized by purging with CO_2 until the pH is below 7. The detailed produce is listed below:

1. In a stainless steel pan, weigh the biomass, lime, and add the distilled water. The loadings are 0.1 g of Ca $(OH)_2$/g dry biomass and 10 mL of distilled water/g dry biomass.
2. Mix the three components very thoroughly to ensure even distribution of the lime and water through the biomass.
3. Place the pan over Bunsen burners and heat to boiling. Boil for 1 h and stir after every 15 min. Add more distilled water if the water content of the mixture is low.
4. Allow the mixture to cool down to room temperature (this usually takes more than 5 h).
5. Once the mixture is cool, add more distilled water to the mixture to cover the biomass. Bubble CO_2 through the mixture using diffusing stones to neutralize the lime. If foaming occurs, add 10 drops of Dow Corning silicone antifoam solution.
6. Continue to bubble CO_2 until the pH falls below 7.0 throughout the biomass. Mix occassionally.
7. Place the pan in the drying oven at 105 °C, and allow the mixture to dry. It may take 2 days. Once the biomass has dried to a solid cake, crumble the cake into pieces by hand and place it in the container to be used.

APPENDIX B

LONG-TERM LIME TREATMENT OF RICE STRAW AND CHICKEN MANURE

Long-term pretreatment was performed by treating biomass with calcium hydroxide (lime) in the presence of water and air at a lower temperature (50^{o}C) for 6 weeks. The ground biomass (80% rice straw, 20% chicken manure), calcium hydroxide (0.2 g/g dry biomass) and water were mixed and placed in a bioreactor (Figure 2-3). The air flow rate through the reactors was measured and the pH was constantly monitored. The detailed procedure is listed below.

1. Mix the biomass (80% rice straw, 20% chicken manure), lime, and distilled water. The loadings are 0.2 g of Ca $(OH)_2$/g dry biomass and 10 mL of distilled water/g dry biomass. Load the mixture into the bioreactor (Figure 2-2).
2. Measure the pH and gas bubbles generated frequently (at least once a week).
3. Allow pretreatment for up to 6 weeks.

APPENDIX C

LIQUID MEDIA PREPARATION

The liquid media used in all fermentation experiments was deoxygenated water with cysteine hydrochloride and sodium sulfide.

1. Pour 5 L of distilled water into a large glass container (6 L).
2. Boil distilled water under a nitrogen purge for 5 min.
3. Cool the boiled water to room temperature under nitrogen purge.
4. Add 0.275 g cysteine hydrochloride and 0.275 g sodium sulfide per liter of boiled distilled water.
5. Stir the solution and pour into storage bottles with a nitrogen purge. Be sure to fill the bottles completely and close the lid tightly.

APPENDIX D
DRY NUTRIENT MIXTURE

The formulation for the dry nutrients mix used in all fermentation experiments was recommended by Ross (1998). The components of the dry nutrients mixture are given in Table E-1.

Table E-1: Dry nutrients mixture

Component	Amount (g/100 g of mixture)
K_2HPO_4	16.3
KH_2PO_4	16.3
NH_2SO_4	16.3
NaCl	32.6
$MgSO_4$ $7H_2O$	6.8
$CaCl_2$ $2H_2O$	4.4
HEPES (N-2-Hydrocyethyl piperazine-N'-2 ethanesulfonate)	0.86
Hemin	0.71
Nicotinamide	0.71
p-Aminobenzoic acid	0.71
Ca-panyothenate	0.71
Folic acid	0.35
Pyrixodal	0.35
Riboflavin	0.35
Thiamin	0.35
Cyanocobalamin	0.14
Biotin	0.14
EDTA	0.35
$FeSO_4$ $7H_2O$	0.14
$MnCl_2$	0.14
H_3BO_3	0.021
$CoCl_2$	0.014
$ZnSO_4$ $7H_2O$	0.007
$NaMoO_4$ $7H_2O$	0.0021
$NiCl_2$	0.0014
$CuCl_2$	0.0007

APPENDIX E
COUNTERCURRENT TRANSFER PROCEDURES

In a countercurrent fermentation, liquid and solid flow in opposite directions in a train of four fermentors. In the laboratory, the transfer of liquid and solids is made every 2 days, operating in a semi-continuous manner. Countercurrent fermentations were initiated as batch fermentations. The experiments were performed in a batch mode until the culture established in the fermentor (~10 days). After this time, the countercurrent operation was started, and the liquid and solid were transferred by using the single-centrifuge procedure. To maintain anaerobic conditions in the fermentors, a nitrogen purge should be utilized every time the fermentors are open to the atmosphere.

SINGLE-CENTRIFUGE PROCEDURE

The single-centrifuge procedure is described below and illustrated in Figure E-1.

1. Remove the fermentors from the incubator and allow cooling for 10 minutes.
2. Measure the gas production using the device illustrated in Figure 2-4.
3. Remove the fermentor caps and place a nitrogen purge line in the fermentor. Using another nitrogen line, remove the residual solids adhered to the stopper and metals bar and returned to the fermentor.
4. Measure and record pH from each fermentor. Place a regular centrifuge cap on the fermentors.
5. Centrifuge the fermentors to separate the solid and the liquid. Pay attention to balance the centrifuge bottles before placing it into the centrifuge. For rice straw and chicken manure fermentations, centrifuge at 4000 rpm for 20 min. After centrifuging, invert the bottles to ensure that the solid and the liquid do not remix.
6. Place the liquid from Fermentor 1 (F1) into a plastic graduate cylinder (previously weighed). Record the weight and volume of liquid.

7. Take a 3-mL liquid sample for carboxylic analysis. Decant the remaining liquid from F1 into a collection bottle for later VS analysis. Store the sample and collection bottle in a freezer analysis.
8. Weigh the fermentor with the remaining solids and compare against the goal weight. To achieve a steady state, a constant wet cake weight is maintained in each fermentor, and then each fermentor is maintained at a specific weight. If the fermentor weight (wet solids + centrifuge bottle without cap) weighs more than the goal weight, remove the difference and the solids aside to be added to Fermentor 2 (F2). To easier the transfer calculations, the goal weight include the desired wet cake weight plus the weight of fresh biomass to be added to F1.
9. Pour the liquid from F2 into F1.
10. Add the fresh biomass to F1 according to the determined loading rate. Add calcium carbonate, urea, dry nutrients, and methane inhibitor. Mix well, replace the stopper and cap the fermentor.
11. Weigh the wet solids from F2. Remove the solids to be removed.
12. Pour the liquid from Fermentor 3 (F3) into F2, and repeat Step 9.
13. Repeat Steps 10 and 11 for F3 and Fermentor 4 (F4).
14. Add fresh liquid medium (Appendix C) to F4 according to predetermined volume.
15. Place the solids removed from F4 in a collection bottle and store it in the freezer until the VS analysis is performed.
16. Return all fermentors back to the incubator.

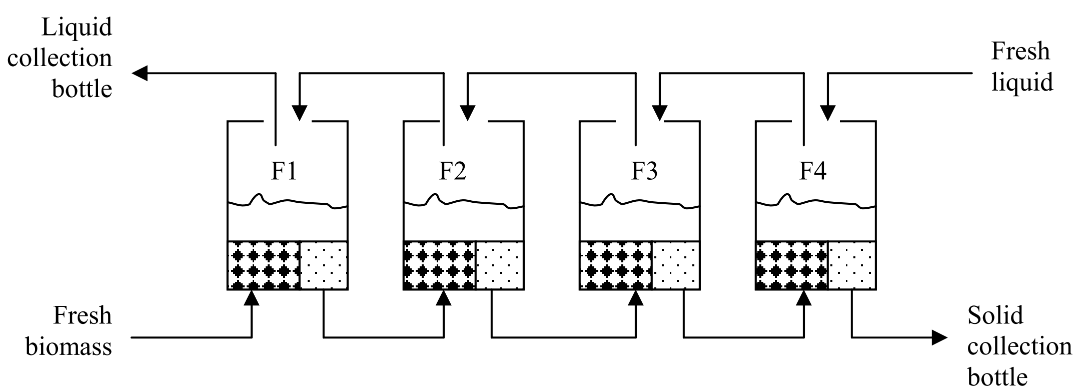


Figure E-1. Single-centrifuge procedure

APPENDIX F
CARBOXYLIC ACIDS ANALYSIS

For carboxylic acids analysis, at least 3 mL of liquid should be withdrawn from the fermentor and placed in a 15-mL conical bottom centrifuge tube. If not used inmediately, the samples may be stored at −15 °C. At the moment of the analysis, if the sample has been stored in the freezer, thaw and vortex the sample before beginning the procedure.

GC LIQUID SAMPLE PREPARATION

1. Centrifuge the liquid sample for 5 min at 3500 rpm.
2. Pipette 1 mL of the liquid clear broth into a 15-mL round-bottom ultracentrifuge tube.
3. Add to the same tube, 1 mL of 10-mM of internal standard 4-methyl-valeric acid (1.162 g/L internal standard, ISTD).
4. Add to the same tube, 1 mL of 3-M phosphoric acid to acidify the sample and allow the carboxylic acids to be released in the GC injection port.
5. Cap the tube and vortex.
6. Centrifuge the mixture at 15,000 rpm (40,000 × g) in the IEC B-20A centrifuge (Industrial Equipment Co., Needham Hts., MA). Due to the poor refrigeration system in the centrifuge, simply accelerate the centrifuge to 15,000 rpm and immediately turn to zero rpm. (Be sure that the temperature is lower than 25ºC before using it).
7. Pipette 1 mL of the centrifugated mixture into a glass GC vial and cap. The sample in the vial is ready to be analyzed. If the sample will not be analyzed immediately, it can be stored in the freezer. If frozen, care should be taken to thaw and vortex the sample before the GC analysis.

GC OPERATION

1. Before starting the GC, check the gas supply cylinders (compressed hydrogen, zero-grade helium, and compressed zero-grade air from Praxair, Bryan, TX) to insure at least 100 psig pressure in each. If there is not enough gas, switch cylinders and place an order for new ones.
2. Establish gas flow by setting the regulators in 40 psig for hydrogen, 60 psig for helium, and 50 psig for air.
3. Check the solvent and waste bottles on the injection tower. Fill the solvent bottles with methanol, and be sure the waste bottles are empty.
4. Make sure the column head pressure gauge on the GC indicates the proper pressure (15 psig). Low head pressure usually indicates a worn-out septum. Replace the septum before starting the GC.
5. Up to 100 samples can be loaded in the autosampler plate. Place the samples in the autosampler racks, not leaving empty spaces between samples. Place volatile acid standard mix (Matreya, Inc. # 1075) solution every 50 samples for calibration.
6. Check the setting conditions in the method:
    a. Oven temperature = 50ºC
    b. Ramp = 20ºC/min
    c. Inlet temperature = 230ºC
    d. Detector temperature = 250ºC
    e. H_2 flow = 40 mL/min
    f. He flow = 180 mL/min
    g. Air flow = 400 mL/min
7. Start the GC on the computer by selecting the method with the setting conditions above mentioned. Set and load the sequence of samples to run. Once the conditions are reached and the green start signal is on the screen, start the run sequence. Details about operation, setting sequence and calibration are in Agilent 6890 instrument manual.

8. Periodically check back to ensure that the equipment is working properly. Be sure to indicate the number of samples and any maintenance performed (changes of septum, gas cylinders, liner, etc.) in the GC logbook.
9. When finish running the sequence, turn the GC on standby and close air and hydrogen cylinder valves.

APPENDIX G
VOLATILE SOLIDS ANALYSIS

PROCEDURE FOR PRODUCT LIQUID

When approximately 900 mL of product liquid have been collected, take the collection bottle out of the freezer and leave the bottle to be thawed overnight. Sometimes, there is a small amount of solid particles in the collected product liquid that were inadvertently washed into the liquid collection bottle. To ensure an accurate measure, this amount of solids also needs to be analyzed for VS, so Steps 10–16 are needed.

1. Record the weight of the full collection bottle (without cap).
2. Centrifuge the liquid collection bottle to separate any solids that might be in the liquid. Use the centrifuge for 20 min at 3500 rpm. When finished, decant all the supernatant liquid into a large clean empty container, being careful not to lose any solids from the bottle.
3. Record the weight of an empty 500-mL Erlenmeyer flask.
4. Add approximately 3 g $Ca(OH)_2$ to the empty container and record weight.
5. Add approximately 100 g of supernatant liquid to the container and record the weight. Mix well. Throw away the rest of the liquid.
6. Record the label and weight of a clean, dry, 150-mL crucible (Crucible A).
7. Pour, while mixing, approximately 70 g of the lime/liquid product mix into Crucible A. Record the weight of the Crucible A + liquid mix.
8. Dry the crucible at 105°C for two days in the drying oven. Place the crucible in a vacuum dessicator and allow it to cool to room temperature before weighing. Record the weight of the crucible.
9. Ash the crucible at 550°C for at least 2 h. Remove the crucible from the ashing oven and place it in a vacuum dessicator and allow it to cool to room temperature. Record the ash weight of the crucible.
10. Record the weight of the collection bottle after pouring off all the liquid.

11. Record the label and weight of a clean, dry, 150-mL crucible (Crucible B).
12. Add approximately 3 g of $Ca(OH)_2$ to Crucible B and record the weight.
13. Mix the remaining content in the liquid collection bottle, and pour carefully approximately 70 g into Crucible B. Mix well the lime and solids, and record the weight of the crucible.
14. Dry the crucible at 105°C as in Step 8.
15. Ash the crucible at 550°C as in Step 9.
16. Wash, dry and record the weight of the empty liquid collection bottle (without cap).

The amount of VS in the supernatant liquid is calculated as

$$\mathrm{VS}_{\text{dissolved}}\,(\mathrm{g\ VS}) = \frac{(\mathrm{W8}-\mathrm{W9})}{\left(\dfrac{\mathrm{W7}-\mathrm{W6}}{\mathrm{W5}-\mathrm{W3}}\right)\times\left(\dfrac{\mathrm{W5}-\mathrm{W4}}{\mathrm{W1}-\mathrm{W10}}\right)}$$

$$\mathrm{VS}_{\text{dissolved}}\,(\mathrm{g\ VS/(g \cdot d)}) = \frac{\dfrac{(\mathrm{W8}-\mathrm{W9})}{\left(\dfrac{\mathrm{W7}-\mathrm{W6}}{\mathrm{W5}-\mathrm{W3}}\right)\times\left(\dfrac{\mathrm{W5}-\mathrm{W4}}{\mathrm{W1}-\mathrm{W10}}\right)}}{\text{collected time period}}$$

The amount of VS in the solid residue present in the liquid is calculated as

$$\mathrm{VS}_{\text{solid residue}}\,(\mathrm{g\ VS}) = \frac{(\mathrm{W14}-\mathrm{W15})}{\left(\dfrac{\mathrm{W13}-\mathrm{W15}}{\mathrm{W10}-\mathrm{W16}}\right)}$$

In all the formulas, W*i* is the weight recorded in the i^{th} step.

PROCEDURE FOR SOLID RESIDUE

1. Record the weight of the full collection bottle (without cap).
2. Empty the solids into a clean empty container, and mix very well. Be careful not to lose any solids from the bottle.
3. Record the label and weight of a clean, dry, 150-mL crucible.
4. Remove a representative sample of approximately 100 g of solid product into the crucible, and record the weight of the crucible.
5. Dry the crucible at 105°C for 2 days in the drying oven. Place the crucible in a vacuum dessicator and allow cooling to room temperature before weighing. Record the dry weight of the crucible.
6. Ash the crucible at 550°C for at least 2 h. Remove quickly the crucible from the ashing oven and place it in a vacuum dessicator and allow cooling to room temperature. Record the ash weight of the crucible.
7. Record the weight of the empty liquid collection bottle (without cap).

The amount of VS in the solid is calculated as

$$VS_{solids} = \frac{(W5 - W6)}{\left(\frac{W4 - W3}{W1 - W7}\right)}$$

The amount of VS in one gram of collected solid is calculated as

$$VS_{\,g\,solid}\ (g\ VS/g\ solids) = \frac{(W5 - W6)}{(W4 - W3)}$$

Again, in all the formulas, Wi represents the weight recorded in the i^{th} step.

APPENDIX H
ACETIC ACID EQUIVALENT

Liquid samples were taken from the fermentors in during batch fermentations as well as during countercurrent fermentations, and were analyzed for carboxylic acids by gas chromatography. In some experiments, the carboxylic acid concentrations were converted into acetic acid equivalents (aceq). The aceq represents the amount of acetic acid that could have been produced in the fermentation if all the carboxylic acids produced were acetic acid (Datta, 1981). The aceq unit is based on the reducing power of the acids produced during the fermentation as presented in the following reducing-power-balanced disproportionation reactions (Loescher, 1996).

Propionic acid:	7 HOAc $\longrightarrow$	HOPr + 2 CO_2 + 2 H_2O	J-1
Butyric acid:	5 HOAc $\longrightarrow$	2 HOBu + 2 CO_2 + 2 H_2O	J-2
Valeric acid:	13 HOAc $\longrightarrow$	4 HOVa + 7 CO_2 + 6 H_2O	J-3
Caproic acid:	4 HOAc $\longrightarrow$	HOCa + 2 CO_2 + 2 H_2O	J-4
Heptanoic acid	19 HOAc $\longrightarrow$	4HOHe + 10 CO_2 + 10 H_2O	J-5

Equations J-1 through J-5 are used to calculate the aceq concentration as expressed in Equation J-6 and J-7

$$\alpha\,(\text{mol/L}) = 1.0\,(\text{acetic}) + 1.75\,(\text{propionic}) + 2.5\,(\text{butyric}) + 3.25\,(\text{valeric}) + 4.0\,(\text{caproic}) + 4.75\,(\text{heptanoic}) \qquad \text{J-6}$$

$$\text{Aceq}\,(\text{g/L}) = 60.05 \times [\text{aceq}\,(\text{mol/L})] \qquad \text{J-7}$$

APPENDIX I

CPDM MATHEMATICA PROGRAM

This appendix contains the CPDM *Mathematica* program used to obtain the predicted product concentration and substrate conversion at various VSLR and LRT. The program results are acid concentration (g acetic acid equivalents/ L) and conversion in each fermentor. The constant values for the system-specific parameters are denoted with "**". VSLR and LRT are the independent variables for constructing the CPDM "map."

```
k=3.5;
While[k<3.51,
l=1;
While[l<1.01,

holdup = 1.4;                     **ratio of liquid to solid in wet cake (g liquid/g VS wet cake)**
moist =0.08;                      **ratio of liquid to solid in feed **
so = 0.6;                         **selectivity, σ (g Aceq/g VS digested)**
ratio = 0.84;                     **ratio of g total acid to g Aceq**
stages = 4;
loading =6;                       **VSLR**
tauloverall = 15*l;               **LRT**
vol = {.34,.17,.17,.17};                   **liquid volume in fermentors**
totvol = Sum[vol[[i]],{i,1,stages}];
liquidfeed = totvol/tauloverall;
nnotreal = {150,150,150,150};;             **VS concentration (g VS/L)**
solidfeed = loading totvol;
Convrsn = {.1,.2,.3,.4};
nnot = nnotreal/(1-Convrsn);
taus = nnot*vol/solidfeed;
L = Table[0.1, {i, 1, stages+1}];
taul = Table[tauloverall/stages, {i, 1, stages}];
fit = {e ->16.312, f->2.749, g->68.124, h ->0.994};            **CPDM parameters**
rmodel[x_, acd_]:= e (1-x)^f/(1+g (acd*ratio)^h)/.fit;         **Eq. (VIII-14)**
rmodel[x, acd];
slp = D[rmodel[x,ac], x];
drmodel[xx_, aac_]:= slp /. {x->xx, ac ->aac};
drmodel[x, ac];
acid =\[InvisibleSpace]{20,10,10,5};
ans=Table[1, {i,1,stages}];
tauloverallnew=20;
taulnew = Table[1000, {i, 1, stages}];
```

```
nhatzero = Table[100, {i, 1, stages}];
done = 0;
liqtoler = 0.05;
acidtoler = 0.02;
nnottoler = 1;
done = 0;
acidold = Table[1.0, {i, 1, stages}];
creation = Table[1, {i, 1, stages}];
destruction = Table[1, {i, 1, stages}];
While[done < 0.50, {taulnew = Table[10000, {j, 1, stages}];
While[Abs[tauloverall-tauloverallnew] > 0.01,
        liquidfeed = liquidfeed (1 + (tauloverallnew-tauloverall)/tauloverall * .5);
        L[[5]] = liquidfeed;
        L[[4]] = L[[5]] + solidfeed/1000  holdup (Convrsn[[4]]-Convrsn[[3]]);
        L[[3]] = L[[4]] + solidfeed/1000 holdup (Convrsn[[3]]-Convrsn[[2]]);
        L[[2]] = L[[3]] + solidfeed/1000 holdup (Convrsn[[2]]-Convrsn[[1]]);
        L[[1]] = moist solidfeed/1000 + L[[2]] - solidfeed/1000 holdup (1.0-Convrsn[[1]]);
        tauloverallnew = totvol/L[[1]]; ];

taul = Table[vol[[j]]/L[[j]], {j, 1, stages}];
scale = Table[1, {j, 1, stages}];
nnot = nnotreal/(1-Convrsn);
taus = nnot*vol/solidfeed;

Print[nnot];
        i=1;

While[Abs[taulnew[[i]] - taul[[i]]] > liqtoler,
        {ans[[i]] = NDSolve[{nhat[0] == 10,
        nhat'[x] == -nhat[x] ( drmodel[x, acid[[i]] ] + so/taus[[i]] )/(rmodel[x, acid[[i]] ])},
        nhat[x], {x, 0, 0.99}];
        factr1 = nnot[[i]]/NIntegrate[ (nhat[x] /. ans[[i]] )[[1]], {x, 0, 0.99}];
        robs = NIntegrate[factr1 (nhat[x] /. ans[[i]] )[[1]] (rmodel[x, acid[[i]] ] ), {x, 0, 0.99}];
        Convrsn[[i]] = NIntegrate[x (nhat[x]/.ans[[1]][[1]]), {x, 0, 0.99}]/nnot[[1]] factr1;
        taulnew[[i]] = (L[[i]] acid[[i]] + solidfeed/1000 (1 - Convrsn[[i]]) holdup acid[[i]] - L[[i+1]]*
        acid[[i+1]])/(L[[i]] robs) ;
        acid[[i]] = acid[[i]] + (taul[[i]] robs -(L[[i]] acid[[i]] + solidfeed/1000 (1 - Convrsn[[i]]) holdup*
        acid[[i]] - L[[i+1]]* acid[[i+1]])/L[[i]] ) 0.4;}];
Print["acid", i, "=", acid[[i]], "  taulnew", i, "=", taulnew[[i]] , "robs =",robs];

        i=2;
        nnottoler = nnot[[i]]/500;
        While[Abs[taulnew[[i]] - taul[[i]]] > liqtoler, {ndone = 0;While[ndone < 0.50,
                {ans[[i]] = NDSolve[{nhat[0] == nhatzero[[i]], nhat'[x] == -nhat[x] ( drmodel[x, acid[[i]]
                ] + so/taus[[i]] )/(rmodel[x, acid[[i]] ]) +(nhat[x]/. ans[[i-1]][[1]] ) nnot[[i]]/nnot[[i-1]]
                factr1 (so/(taus[[i]] rmodel[x, acid[[i]] ] ) ) },nhat[x], {x, 0, 0.99}];

                nhattot = NIntegrate[(nhat[x]/. ans[[i]])[[1]], {x, 0, 0.99}];
                Print["nhatzero=", nhatzero[[i]], " nhattot=", nhattot, "nnot[[i]]=",nnot[[i]] ];
                ndone = If[Abs[nhattot - nnot[[i]] ] < nnottoler, 1, 0];
                nhatzero[[i]] = If[nhatzero[[i]] + (nnot[[i]] - nhattot) 1.0 > 0,
                nhatzero[[i]]  + (nnot[[i]] - nhattot)/nnot[[i]] 50 ,
```

```
                nhatzero[[i]]  + (nnot[[i]] - nhattot)/nnot[[i]] 50 ] } ];

                Convrsn[[i]] = (NIntegrate[x (nhat[x]/.ans[[i]][[1]]), {x, 0, 0.99}])/nnot[[i]];
                robs = solidfeed so/vol[[i]] (Convrsn[[i]] - Convrsn[[i-1]]);
                 taulnew[[i]] = (L[[i]] acid[[i]] + solidfeed/1000 (1 - Convrsn[[i]] ) holdup acid[[i]]
                - L[[i+1]] acid[[i+1]] -solidfeed/1000 (1 - Convrsn[[i-1]] ) holdup acid[[i-1]])/
                (L[[i]] robs);
                acid[[i]] = acid[[i]] + (taul[[i]] robs - (L[[i]] acid[[i]] + solidfeed/1000
                (1 - Convrsn[[i]] ) holdup acid[[i]] - L[[i+1]] acid[[i+1]] -
                solidfeed/1000 (1 - Convrsn[[i-1]] ) holdup acid[[i-1]])/L[[i]]) 0.5;}];

        Print["acid", i, "=", acid[[i]], "  taulnew", i, "=", taulnew[[i]] , "robs =",robs];

        i=3;
        nnottoler = nnot[[i]]/500;
        While[Abs[taulnew[[i]] - taul[[i]]] > liqtoler,
                {ndone = 0;
                While[ndone < 0.50,
                {ans[[i]] = NDSolve[{nhat[0] == nhatzero[[i]],
                nhat'[x] == -nhat[x] ( drmodel[x, acid[[i]] ] + so/taus[[i]] )/(rmodel[x, acid[[i]] ]) +
                (nhat[x]/. ans[[i-1]][[1]] ) nnot[[i]]/nnot[[i-1]] (so/(taus[[i]] rmodel[x, acid[[i]] ] ) ) },
                nhat[x], {x, 0, 0.99}];
                nhattot = NIntegrate[(nhat[x]/. ans[[i]])[[1]], {x, 0, 0.99}];
                Print["nhatzero=", nhatzero[[i]], " nhattot=", nhattot, "nnot[[i]]=",nnot[[i]] ];
                ndone = If[Abs[nhattot - nnot[[i]] ] < nnottoler, 1, 0];

                nhatzero[[i]] = If[nhatzero[[i]] + (nnot[[i]] - nhattot) 1.0 > 0,
                nhatzero[[i]]  + (nnot[[i]] - nhattot)/nnot[[i]] 25 ,
                nhatzero[[i]]  + (nnot[[i]] - nhattot)/nnot[[i]] 25 ] } ];

                Convrsn[[i]] = (NIntegrate[x (nhat[x]/.ans[[i]][[1]]), {x, 0, 0.99}])/nnot[[i]];
                        robs = solidfeed so/vol[[i]] (Convrsn[[i]] - Convrsn[[i-1]]);
                Convrsn[[i]] = (NIntegrate[x (nhat[x]/.ans[[i]][[1]]), {x, 0, 0.99}])/nnot[[i]];
                taulnew[[i]] = (L[[i]] acid[[i]] + solidfeed/1000 (1 - Convrsn[[i]] ) holdup acid[[i]]
                - L[[i+1]] acid[[i+1]] -solidfeed/1000 (1 - Convrsn[[i-1]] ) holdup acid[[i-1]])/
                (L[[i]] robs);
                acid[[i]] = acid[[i]] + (taul[[i]] robs - (L[[i]] acid[[i]] + solidfeed/1000
                (1 - Convrsn[[i]] ) holdup acid[[i]] - L[[i+1]] acid[[i+1]] -
                solidfeed/1000 (1 - Convrsn[[i-1]] ) holdup acid[[i-1]])/L[[i]]) 0.5;} ];

Print[" acid", i, "=", acid[[i]], "  taulnew", i, "=", taulnew[[i]] , "robs=",robs];

        i = 4;
        nnottoler = nnot[[i]]/500;
        scale[[4]]=0.5;
        While[Abs[taulnew[[i]] - taul[[i]]] > liqtoler,
                {ndone = 0;
                While[ndone < 0.50,
                {ans[[i]] = NDSolve[{nhat[0] == nhatzero[[i]],
                nhat'[x] == -nhat[x] ( drmodel[x, acid[[i]] ] + so/taus[[i]] )/(rmodel[x, acid[[i]] ]) +
                (nhat[x]/. ans[[i-1]][[1]] ) nnot[[i]]/nnot[[i-1]] (so/(taus[[i]] rmodel[x, acid[[i]] ] ) ) },
                nhat[x], {x, 0, 0.99}];
```

```
                    nhattot = NIntegrate[(nhat[x]/. ans[[i]])[[1]], {x, 0, 0.99}];
                    Print["nhatzero=", nhatzero[[i]], " nhattot=", nhattot, "nnot[[i]]=",nnot[[i]] ];

                    ndone = If[Abs[nhattot - nnot[[i]] ] < nnottoler, 1, 0];

                    nhatzero[[i]] = If[nhatzero[[i]] + (nnot[[i]] - nhattot) 1.0 > 0,
                    nhatzero[[i]]  + (nnot[[i]] - nhattot)/nnot[[i]] 25 ,
                    nhatzero[[i]]  + (nnot[[i]] - nhattot)/nnot[[i]] 25 ] } ];
                    Convrsn[[i]] = (NIntegrate[x (nhat[x]/.ans[[i]][[1]]), {x, 0, 0.99}])/nnot[[i]];
                    robs = solidfeed so/vol[[i]] (Convrsn[[i]] - Convrsn[[i-1]]);
                    taulnew[[i]] = (L[[i]] acid[[i]] + solidfeed/1000 (1 - Convrsn[[i]] ) holdup acid[[i]]
                     -solidfeed/1000 (1 - Convrsn[[i-1]] ) holdup acid[[i-1]])/(L[[i]] robs);
                    acid[[i]] = acid[[i]] + (taul[[i]] robs - (L[[i]] acid[[i]] + solidfeed/1000
                    (1 - Convrsn[[i]] ) holdup acid[[i]] -
                    solidfeed/1000 (1 - Convrsn[[i-1]] ) holdup acid[[i-1]])/L[[i]]) 0.5;} ];

Print["  acid", i, "=", acid[[i]], "  taulnew", i, "=", taulnew[[i]] , "robs =",robs];

Convrsn=Flatten[{NIntegrate[x (nhat[x]/.ans[[1]][[1]]), {x, 0, 0.99}]/nnot[[1]] factr1,
        Table[(NIntegrate[x (nhat[x]/.ans[[i]][[1]]), {x, 0, 0.99}])/nnot[[i]],
       i,2,stages}]}];Print["conversion in each stage (from nhat)", Convrsn];

done = If[Max[Abs[(acidold-acid)] ] < acidtoler, 1, 0]; acidold = acid}]
Print[L[[1]]];
Print[L[[2]]];
Print[L[[3]]];
Print[L[[4]]];
Print[L[[5]]];
creation[[1]] = L[[1]] acid[[1]] + solidfeed/1000 (1 - Convrsn[[1]]) holdup acid[[2]] - L[[2]] acid[[2]] ;
creation[[2]] = L[[2]] acid[[2]] + solidfeed/1000 (1 - Convrsn[[2]]) holdup acid[[3]] - L[[3]] acid[[3]] -
                solidfeed/1000 (1 - Convrsn[[1]]) holdup acid[[2]];
creation[[3]] = L[[3]] acid[[3]] + solidfeed/1000 (1 - Convrsn[[3]]) holdup acid[[4]] - L[[4]] acid[[4]] -
solidfeed/1000 (1 - Convrsn[[2]]) holdup acid[[3]];
creation[[4]] = L[[4]] acid[[4]]  -   solidfeed/1000 (1 - Convrsn[[3]]) holdup acid[[4]];
destruction[[1]] = solidfeed/1000 (Convrsn[[1]] - 0);
destruction[[2]] = solidfeed/1000 (Convrsn[[2]] - Convrsn[[1]]);
destruction[[3]] = solidfeed/1000 (Convrsn[[3]] - Convrsn[[2]]);
destruction[[4]] = solidfeed/1000 (Convrsn[[4]] - Convrsn[[3]]);

Print["Selectivity = ",creation/destruction];
Print["Creation = ", creation];
Print["destruction = ",destruction];
selec = L[[1]] acid[[1]]/(solidfeed Convrsn[[4]]);
Print["selectivity = ",selec];
Print["k = ",k,"   l = ",l];
Print["loading = ", loading];
Print["tauloverall  ", tauloverall];
Print["taus  ", Sum[taus[[i]], {i, 1, stages}]];
Print["acid levels  ",acid];
l = l + 0.5;];
k = k + 0.5;];
```

APPENDIX J

CPDM MATLAB PROGRAM FOR FIXED-BED FERMENTORS

```
% Fixed-bed modeling
%Reactor 1
clear
%V is total volume of liquid in each fermentors (L)
V=0.514;
%d is the number of days for each transfer transfer
d=4;
%Setup total number of transfers
n=77;
%Set up number of points for batch data
b=8;
%Seup liquid volume (L) transferred q
q=0.010;
%Enter VS fed(g)
s=44;
%Set up space for time, t, Acid mass A, Acetic concentration, C, Total
acid T
t=zeros(n,1);
A1=zeros(n,1);
C1=zeros(n,1);
T1=zeros(n,1);
p=zeros(n,1);
%Initial total acid in A
i=1;
A1(i,1)=3.7303;
p(i,1)=0.8492;
%Calculate acid concentration
C1(i,1)=A1(i,1)/V;
T1(i,1)=C1(i,1).*p(i,1);
e=0.0342;
f=2.299;
g=1.36e-4;
h=1.97;
%Run calculations for batch mode in F1
while i <b,
%Enter the value of phi ,p
x=A1(i,1)/s;
r1=e*((1-x)^f)/(1+g*((p(i,1).*A1(i,1))^h));
A1(i+1,1)=A1(i,1)+r1*d*s-0.003.*A1(i,1)./V;
t(i+1,1)=t(i,1)+d;
p(i+1,1)=1E-05.*(t(i+1,1)^2)-0.0031.*t(i+1,1)+ 0.7824;
i=i+1;
C1(i,1)=A1(i,1)./V;
T1(i,1)=C1(i,1).*p(i,1);
end
%Calculate for liquid movement in F1
while i < n
   while x<0.9
```

```
   r1=e*((1-x)^f)/(1+g*((p(i,1).*A1(i,1))^h));
   A1(i+1,1)=A1(i,1)-((q+0.003).*C1(i,1))+r1*d*s;
   x=x+(r1.*d)+((q+0.003).*C1(i,1)/s);
   t(i+1,1)=t(i,1)+d;
   p(i+1,1)=1E-05.*(t(i+1,1)^2)-0.0031.*t(i+1,1)+ 0.7824;
   i=i+1;
   C1(i,1)=A1(i,1)./V;
   T1(i,1)=C1(i,1).*p(i,1);
   t1=max(t);
   end
A1(i+1,1)=A1(i,1)-((q+0.003).*C1(i,1));
t(i+1,1)=t(i,1)+d;
p(i+1,1)=1E-05.*(t(i+1,1)^2)-0.0031.*t(i+1,1)+ 0.7824;
i=i+1;
C1(i,1)=A1(i,1)./V;
T1(i,1)=C1(i,1).*p(i,1);
end
MA=T1';
[TA,i]=max(MA);
MA1=[T1,t];
mas1=MA1(i,:)
load Set1a.m;
s1=Set1a(:,1);
s2=Set1a(:,2);
figure(1)
plot(s1,s2,'k.',t,T1,'k-')
xlabel('Time (days)')
ylabel('Total acid concentration (g/L)')
c1=corrcoef(s2,T1)


t=zeros(n,1);
A2=zeros(n,1);
C2=zeros(n,1);
T2=zeros(n,1);
p=zeros(n,1);
%Initial total acid in A
i=1
A2(i,1)=3.7303;
%Calculate acid concentration
C2(i,1)=A2(i,1)/V;
p(i,1)=0.8506;
T2(i,1)=C2(i,1).*p(i,1);
%Run calculations for batch mode in F1
while i <b,
%Enter the value of phi ,p
x=A2(i,1)/s;
r1=e*((1-x)^f)/(1+g*((p(i,1).*A2(i,1))^h));
A2(i+1,1)=A2(i,1)+r1*d*s-0.003.*A2(i,1)./V;
t(i+1,1)=t(i,1)+d;
p(i+1,1)=2E-06.*(t(i+1,1)^2)-0.0011.*t(i+1,1)+ 0.8179;
i=i+1;
C2(i,1)=A2(i,1)./V;
```

```
T2(i,1)=C2(i,1).*p(i,1);
end
%Calculate for liquid movement in F1
while i < n
   while x<0.9
   r1=e*((1-x)^f)/(1+g*((p(i,1).*A2(i,1))^h));
   A2(i+1,1)=A2(i,1)-((q+0.003).*C2(i,1))+r1*d*s+(q.*C1(i,1));
   x=x+(r1.*d)+((q+0.003).*C2(i,1)/s)-(q.*C1(i,1)/s);
   t(i+1,1)=t(i,1)+d;
   p(i+1,1)=2E-06.*(t(i+1,1)^2)-0.0011.*t(i+1,1)+ 0.8179;
   i=i+1;
   C2(i,1)=A2(i,1)./V;
   T2(i,1)=C2(i,1).*p(i,1);
   t2=max(t);
   end
A2(i+1,1)=A2(i,1)-((q+0.003).*C2(i,1))+(q.*C1(i,1));
t(i+1,1)=t(i,1)+d;
p(i+1,1)=2E-06.*(t(i+1,1)^2)-0.0011.*t(i+1,1)+ 0.8179;
i=i+1;
C2(i,1)=A2(i,1)./V;
T2(i,1)=C2(i,1).*p(i,1);
end
MA=T2';
[TA,i]=max(MA);
MA2=[T2,t];
mas2=MA2(i,:)
load Set1a.m;
s1=Set1a(:,1);
s2=Set1a(:,3);
figure(2)
plot(s1,s2,'k.',t,T2,'k-')
xlabel('Time (days)')
ylabel('Total acid concentration (g/L)')
c2=corrcoef(s2,T2)

t=zeros(n,1);
A3=zeros(n,1);
C3=zeros(n,1);
T3=zeros(n,1);
p=zeros(n,1);
%Initial total acid in A
i=1;
A3(i,1)=3.7303;
%Calculate acid concentration
C3(i,1)=A3(i,1)/V;
p(i,1)=0.726;
T3(i,1)=C3(i,1).*p(i,1);
%Run calculations for batch mode in F1
while i <b,
%Enter the value of phi ,p
x=A3(i,1)/s;
r1=e*((1-x)^f)/(1+g*((p(i,1).*A3(i,1))^h));
A3(i+1,1)=A3(i,1)+r1*d*s-0.003.*A3(i,1)./V;
```

```
t(i+1,1)=t(i,1)+d;
p(i+1,1)=0.726;
i=i+1;
C3(i,1)=A3(i,1)./V;
T3(i,1)=C3(i,1).*p(i,1);
end
%Calculate for liquid movement in F1
while i < n
   while x<0.9
   r1=e*((1-x)^f)/(1+g*((p(i,1).*A3(i,1))^h));
   A3(i+1,1)=A3(i,1)-((q+0.003).*C3(i,1))+r1*d*s+(q.*C2(i,1));
   x=x+(r1.*d)+((q+0.003).*C3(i,1)/s)-(q.*C2(i,1)/s);
   t(i+1,1)=t(i,1)+d;
   p(i+1,1)=0.726;
   i=i+1
   C3(i,1)=A3(i,1)./V;
   T3(i,1)=C3(i,1).*p(i,1);
   t3=max(t);
   end
A3(i+1,1)=A3(i,1)-((q+0.003).*C3(i,1))+(q.*C2(i,1));
t(i+1,1)=t(i,1)+d;
p(i+1,1)=0.726;
i=i+1;
C3(i,1)=A3(i,1)./V;
T3(i,1)=C3(i,1).*p(i,1);
end
MA=T3';
[TA,i]=max(MA);
MA3=[T3,t];
mas3=MA3(i,:)
load Set1a.m;
s1=Set1a(:,1);
s2=Set1a(:,4);
figure(3)
plot(s1,s2,'k.',t,T3,'k-')
xlabel('Time (days)')
ylabel('Total acid concentration (g/L)')
c3=corrcoef(s2,T3)

t=zeros(n,1);
A4=zeros(n,1);
C4=zeros(n,1);
T4=zeros(n,1);
p=zeros(n,1);
%Initial total acid in A
i=1;
A4(i,1)=3.7303;
%Calculate acid concentration
C4(i,1)=A4(i,1)/V;
p(i,1)=0.745;
T4(i,1)=C4(i,1).*p(i,1);
%Run calculations for batch mode in F1
while i <b,
```

```
%Enter the value of phi ,p
x=A4(i,1)/s;
r1=e*((1-x)^f)/(1+g*((p(i,1).*A4(i,1))^h));
A4(i+1,1)=A4(i,1)+r1*d*s-0.003.*A4(i,1)./V;
t(i+1,1)=t(i,1)+d
p(i+1,1)=0.745;
i=i+1;
C4(i,1)=A4(i,1)./V;
T4(i,1)=C4(i,1).*p(i,1);
end
%Calculate for liquid movement in F4
while i < n
   while x<0.9
   r1=e*((1-x)^f)/(1+g*((p(i,1).*A4(i,1))^h));
   A4(i+1,1)=A4(i,1)-((q+0.003).*C4(i,1))+r1*d*s+(q.*C3(i,1));
   x=x+(r1.*d)+((q+0.003).*C4(i,1)/s)-(q.*C3(i,1)/s);
   t(i+1,1)=t(i,1)+d;
   p(i+1,1)=0.745;
   i=i+1;
   C4(i,1)=A4(i,1)./V;
   T4(i,1)=C4(i,1).*p(i,1);
   t4=max(t)
   end
A4(i+1,1)=A4(i,1)-((q+0.003).*C4(i,1))+(q.*C3(i,1));
t(i+1,1)=t(i,1)+d;
p(i+1,1)=0.745;
i=i+1;
C4(i,1)=A4(i,1)./V;
T4(i,1)=C4(i,1).*p(i,1);
end
MA=T4';
[TA,i]=max(MA);
MA4=[T4,t];
mas4=MA4(i,:)
load Set1a.m;
s1=Set1a(:,1);
s2=Set1a(:,5);
figure(4)
plot(s1,s2,'k.',t,T4,'k-')
xlabel('Time (days)')
ylabel('Total acid concentration (g/L)')
%ma=[ma1;ma2;ma3;ma4]
TF=[t1,t2,t3,t4]
mas=[mas1;mas2;mas3;mas4]
c4=corrcoef(s2,T4)
c1,c2,c3,c4
```

APPENDIX K

CPDM MATLAB PROGRAM FOR ROUND ROBIN FERMENTORS

```
% Round robin
clear
%V is total volume of liquid in fermentors (L)
V=0.514;
%d is the number of days for each transfer transfer
d=4;
%Setup total number of transfers
n=150;
%Set up number of points for batch data
b=8;
%Setup liquid volume (L) transferred q
q=0.10;
%Enter VS fed(g)
s=44;
%Set up space for time, t, Acid mass A, Acetic concentration, C, Total
acid T
t=zeros(n,1);
A=zeros(n,5);
C=zeros(n,5);
T=zeros(n,5);
p=zeros(n,5);
x=zeros(n,5);
r=zeros(n,5);
%Initial total acid in A
i=1;
A(i,1)=3.7303;A(i,2)=3.7303;A(i,3)=3.7303;A(i,4)=3.7303;A(i,5)=3.7303;
p(i,1)=0.8492;p(i,2)=0.8506;p(i,3)=0.726;p(i,4)=0.745;p(i,5)=0.745;
%Calculate acid concentration
C(i,1)=A(i,1)/V;C(i,2)=A(i,2)/V;C(i,3)=A(i,3)/V;C(i,4)=A(i,4)/V;C(i,5)=
A(i,5)/V;
T(i,1)=C(i,1).*p(i,1);T(i,2)=C(i,2).*p(i,2);T(i,3)=C(i,3).*p(i,3);T(i,4
)=C(i,4).*p(i,4);T(i,5)=C(i,5).*p(i,5);
e=0.0342;
f=2.299;
g=1.33e-4;
h=1.97;
%Run calculations for batch mode in F1
while i <b,
   for j=1:5
   x(i,j)=A(i,j)/s;
   r(i,j)=e*((1-x(i,j))^f)/(1+g*((p(i,j).*A(i,j))^h));
   A(i+1,j)=A(i,j)+r(i,j)*d*s-0.003.*A(i,j)./V;
   end
t(i+1,1)=t(i,1)+d;
p(i+1,1)=0.726;
p(i+1,2)=0.726;
p(i+1,3)=0.726;
p(i+1,4)=0.726;
```

```
p(i+1,5)=0.726;

i=i+1;
C(i,1)=A(i,1)/V;C(i,2)=A(i,2)/V;C(i,3)=A(i,3)/V;C(i,4)=A(i,4)/V;C(i,5)=
A(i,5)/V;
T(i,1)=C(i,1).*p(i,1);T(i,2)=C(i,2).*p(i,2);T(i,3)=C(i,3).*p(i,3);T(i,4
)=C(i,4).*p(i,4);T(i,5)=C(i,5).*p(i,5);
x(i,1)=A(i,1)/s; x(i,2)=A(i,2)/s; x(i,3)=A(i,3)/s;
x(i,4)=A(i,4)/s;x(i,5)=A(i,5)/s;

end
a1=x(i,1);m=x(i,2);u=x(i,3);v=x(i,4);a2=x(i,5);
A1=A(i,j);
Rrobinn
while i < n
   j=1;
   x(i,j)=x(i,j+1)+(C(i,j+1).*q/s);
   A(i,j)=A(i,j+1)-C(i,j+1).*q;
   C(i,j)=A(i,j)/V;
   T(i,j)=C(i,j).*p(i,j);
   for j=2:4
   x(i,j)=x(i,j+1)+((C(i,j+1)-C(i,j)).*q/s);
   A(i,j)=A(i,j+1)+(C(i,j)-C(i,j+1)).*q;
   C(i,j)=A(i,j)/V;
   T(i,j)=C(i,j).*p(i,j);
   end

   j=5;
   A(i,j)=A1+C(i,j).*q;
   C(i,j)=A(i,j)/V;
   x(i,j)=A(i,j)/s;
   a=x(i,1);
   Rrobinn1
end

plot(t,T(:,5),'k')
xlabel('Time (days)')
ylabel('Total acid concentration (g/L)')


Rrobinn

while a1 < 0.9
      j=1;
      r(i,j)=e*((1-x(i,j))^f)/(1+g*((p(i,j).*A(i,j))^h));
      A(i+1,j)=A(i,j)-((q+0.003).*C(i,j))+r(i,j)*d*s;
      x(i+1,j)=x(i,j)+(r(i,j).*d)+((q+0.003).*C(i,j)./s);
      for j=2:5
      r(i,j)=e*((1-x(i,j))^f)/(1+g*((p(i,j).*A(i,j))^h));
      A(i+1,j)=A(i,j)-((q+0.003).*C(i,j))+r(i,j)*d*s+(q.*C(i,j-1));
      x(i+1,j)=x(i,j)+(r(i,j).*d)+((q+0.003).*C(i,j)/s)-(q.*C(i,j-
1)./s);
      end
```

```
      t(i+1,1)=t(i,1)+d;
      p(i+1,1)=0.726;
      p(i+1,2)=0.726;
      p(i+1,3)=0.726;
      p(i+1,4)=0.726;
      p(i+1,5)=0.726;

      i=i+1;

   C(i,1)=A(i,1)/V;C(i,2)=A(i,2)/V;C(i,3)=A(i,3)/V;C(i,4)=A(i,4)/V;C(i,
5)=A(i,5)/V;

   T(i,1)=C(i,1).*p(i,1);T(i,2)=C(i,2).*p(i,2);T(i,3)=C(i,3).*p(i,3);T(
i,4)=C(i,4).*p(i,4);T(i,5)=C(i,5).*p(i,5);
      a1=x(i,1);
  end

Rrobinn1
while a < 0.9
      j=1;
      r(i,j)=e*((1-x(i,j))^f)/(1+g*((p(i,j).*A(i,j))^h));
      A(i+1,j)=A(i,j)-((q+0.003).*C(i,j))+r(i,j)*d*s;
      x(i+1,j)=x(i,j)+(r(i,j).*d)+((q+0.003).*C(i,j)./s);
      for j=2:5
      r(i,j)=e*((1-x(i,j))^f)/(1+g*((p(i,j).*A(i,j))^h));
      A(i+1,j)=A(i,j)-((q+0.003).*C(i,j))+r(i,j)*d*s+(q.*C(i,j-1));
      x(i+1,j)=x(i,j)+(r(i,j).*d)+((q+0.003).*C(i,j)/s)-(q.*C(i,j-
1)./s);
      end
      t(i+1,1)=t(i,1)+d;
      p(i+1,1)=0.726;
      p(i+1,2)=0.726;
      p(i+1,3)=0.726;
      p(i+1,4)=0.726;
      p(i+1,5)=0.726;

      i=i+1;

   C(i,1)=A(i,1)/V;C(i,2)=A(i,2)/V;C(i,3)=A(i,3)/V;C(i,4)=A(i,4)/V;C(i,
5)=A(i,5)/V;

   T(i,1)=C(i,1).*p(i,1);T(i,2)=C(i,2).*p(i,2);T(i,3)=C(i,3).*p(i,3);T(
i,4)=C(i,4).*p(i,4);T(i,5)=C(i,5).*p(i,5);
      a=x(i,1);
 end
```

APPENDIX L

DETERMINATION OF LIGNIN (ACID SOLUBLE AND ACID INSOLUBLE)

1. Wash biomass and dry at 45^{o}C overnight.
2. Determine moisture content of biomass.
3. Weigh 0.3 g of prepared sample to the nearest 0.1 mg and place in 16×100 mm test tube. Record as W_1, the initial weight.
4. Add 3 mL of 72% H_2SO_4 and use a glass stirring rod to mix for 1 minute, or until the sample is thoroughly wetted.
5. Stir the sample every 15 min for 2 h at room temperature to assure complete mixing and wetting.
6. Transfer the hydrolyzate to a glass bottle and dilute to 4% acid concentration by adding 84 mL water. Be careful to transfer all the residual solids along with the hydrolysis liquor.
7. Stopper each of the bottles and crimp aluminium seals in place.
8. Set the autoclave to a liquid vent cycle to prevent loss of sample from the bottle in the event of loose crimp seal. Autoclave the samples in their sealed bottles for 1 h at 121^{o}C.
9. After completion of the autoclave cycle, allow the samples to cool for about 20 min at room temperature before removing the seals and stoppers.
10. Vacuum filter the hydrolysis solution through one of the previously ignited filtering crucibles.
11. If carbohydrate analysis and/or acid soluble lignin analysis is desired, decant 15–25 mL of filterate into a resealable container. If the aliquot is not used immediately for further analysis, store in refrigerator at 4^{o}C. Acid-soluble lignin should be analyzed within 24 h, preferably within 6 h of hydrolysis.
12. Use hot deionized water to wash any particles clinging to the glass bottle into the crucible and to wash the filtered residue free of acid using vacuum.
13. Dry the crucible and contents at 105^{o}C for 2 h or until contant weight is achieved.

14. Cool in desiccator and record the weight, W_2, the weight of the crucible, acid-insoluble lignin, and acid-insoluble ash to the nearest 0.1 mg.
15. Place the crucible and contents in the muffle furnace and ignite at 575°C for a minimum of 3 h.
16. Cool in desiccator and record the weight, W_3, the weight of the crucible and acid-insoluble ash.

$$\text{\% Acid-insoluble lignin} = \frac{(W_2 - W_3)}{\left(W_1 \times \frac{T_{final}}{100}\right)} \times 100\%$$

where W_1 = initial sample weight

W_2 = weight of crucible, acid-insoluble lignin, and acid-insoluble ash

W_3 = weight of crucible, and acid-insoluble ash

T_{final} = % total solids content of the prepared sample used in this lignin analysis, on a 105°C dry weight basis.

17. Dilute the filterate to obtain absorbance between 0.2 and 0.7.
18. Measure the absorbance of the filterate at 205 nm. Use 4% H_2SO_4 solution as a reference blank.
19. The percent acid soluble lignin on a 105°C dry weight basis is given as

$$\text{\% Acid soluble lignin} = \frac{\left(\frac{A}{b \times a} \times df \times V \times \frac{\text{L}}{1000\,\text{mL}}\right)}{\left(W_1 \times \frac{T_{final}}{100}\right)} \times 100\%$$

where A = absorbance at 205 nm

df = dilution factor

b = cell length, 1 cm

a = absorptivity, equal to 110 L/g-cm

V = filterate volume, 87 mL

W = initial biomass sample weight in grams

$\%T_{final}$ = % total solids content of biomass sample on a 105°C dry weight basis

APPENDIX M

GLUCOSE EQUIVALENT BY DINITROSALICYLIC ACID ASSAY (DNS)

1. Prepare dilutions of known glucose concentration in test tubes using glucose standard. Centrifuge hydrolysis samples at 15,000 rpm to clarify the liquid.
2. Add 3 mL of DNS to 0.5 mL of sample.
3. Place caps on tubes and vortex.
4. Boil samples in a water bath for 5 min.
5. Cool the test tubes to room temperature and add 10 mL of distilled water. Place caps and vortex.
6. Allow the spectrophometer to warm up for at least 30 min. Zero the spectrophotometer (Spectronic 1001) at 540 nm with distilled water.
7. Measure the absorbance immediately.

APPENDIX N

Table N3A. Fermentation data for Fermentation Train A

Day	ave pH	VS in (g)	liq out (mL)	solid out (g)	acid out (g)	VS solid out (g)	VS liq out (g)	VS residue (g)	Biotic CO_2 (g)	CH_4 (g)	Total gas out (g)	Abiotic CO_2 (g)
8	5.56	13.92	220.0	0.0	4.89	0.00	15.05	0	1.041	0.000	3.330	2.289
12	5.63	13.92	189.0	0.0	7.02	0.00	12.93	0	0.134	0.000	2.100	1.967
14	5.60	13.92	173.5	0.0	6.05	0.00	11.87	0	0.020	0.000	1.818	1.798
16	5.80	13.92	197.0	0.0	6.70	0.00	13.47	0	0.035	0.000	2.085	2.050
18	5.87	13.92	74.0	0.0	2.53	0.00	5.06	0	0.124	0.000	0.894	0.770
22	5.96	13.92	21.5	68.0	0.75	0.40	1.47	11.4	1.250	0.000	1.474	0.224
24	5.90	13.92	164.0	0.0	6.01	0.00	11.22	0	0.012	0.000	1.718	1.706
30	5.91	13.92	130.0	0.0	4.36	0.00	8.89	0	0.205	0.000	1.558	1.353
32	5.87	13.92	45.0	58.0	1.63	0.34	3.08	9.74	0.380	0.000	0.848	0.468
34	5.98	13.92	68.0	85.0	2.31	0.50	4.65	14.3	0.423	0.000	1.130	0.708
36	5.76	13.92	103.0	0.0	3.54	0.00	7.05	0	0.078	0.000	1.149	1.072
38	5.76	13.92	120.0	18.0	4.12	0.11	8.21	3.02	0.004	0.000	1.253	1.249
40	5.76	13.92	120.0	18.0	4.38	0.11	8.21	3.02	1.203	0.000	2.452	1.249
50	5.71	13.92	89.7	24.2	3.27	0.14	6.14	4.69	1.475	0.009	2.417	0.933
52	5.80	13.92	85.2	52.6	3.02	0.31	5.83	10.2	0.036	0.009	0.932	0.887
54	5.85	13.92	52.8	34.7	1.81	0.20	3.61	6.73	0.507	0.009	1.065	0.549
60	5.93	13.92	68.0	74.0	2.38	0.44	4.65	14.4	4.924	0.009	5.640	0.708
63	5.90	13.92	111.0	2.0	3.93	0.01	7.59	0.39	1.876	0.009	3.040	1.155
65	5.99	13.92	80.0	74.0	2.85	0.44	5.47	14.4	2.397	0.009	3.238	0.832
67	6.05	13.92	54.0	79.0	1.86	0.46	3.69	15.3	0.177	0.009	0.748	0.562
69	5.93	13.92	82.0	33.0	2.84	0.19	5.61	6.4	0.127	0.009	0.989	0.853
73	6.00	13.92	126.0	7.0	4.36	0.04	8.62	1.36	0.200	0.009	1.520	1.311
75	6.00	13.92	108.0	52.0	3.47	0.31	7.39	10.1	0.417	0.009	1.550	1.124
77	5.94	13.92	124.0	14.0	4.09	0.08	8.48	2.72	0.549	0.009	1.848	1.290
79	5.96	13.92	75.0	103.0	2.42	0.61	5.13	20	0.978	0.009	1.768	0.780
81	5.94	13.92	108.0	0.0	3.60	0.00	7.39	0	0.563	0.009	1.696	1.124
83	5.99	13.92	77.0	29.0	2.66	0.17	5.27	5.63	1.183	0.009	1.993	0.801
85	5.96	13.92	53.0	22.0	1.81	0.13	3.63	4.27	1.276	0.009	1.837	0.551
87	6.06	13.92	64.0	60.0	2.19	0.35	4.38	11.6	1.662	0.009	2.337	0.666
89	6.11	13.92	55.0	108.0	1.88	0.64	3.76	21	0.014	0.009	0.596	0.572
91	5.96	13.92	78.0	0.0	2.53	0.00	5.34	0	1.799	0.009	2.620	0.812
95	6.05	13.92	67.0	49.0	2.38	0.29	4.58	9.51	1.738	0.009	2.444	0.697
101	6.12	13.92	54.0	5.0	1.98	0.03	3.69	0.97	3.542	0.009	4.113	0.562
105	5.94	13.92	111.0	25.0	4.33	0.15	7.59	4.85	1.494	0.009	2.658	1.155
136	5.89	13.92	146.0	0.0	5.86	0.00	9.99	0	1.683	0.009	3.212	1.519
138	6.32	13.92	65.0	39.0	2.36	0.23	4.45	7.57	0.659	0.009	1.344	0.676
140	6.26	13.92	60.0	58.0	2.16	0.34	4.10	11.3	0.611	0.009	1.245	0.624
142	6.22	13.92	81.0	45.0	2.78	0.26	5.54	8.73	1.042	0.009	1.894	0.843
144	6.27	13.92	66.0	51.0	2.32	0.30	4.51	9.89	1.198	0.009	1.894	0.687

146	6.24	13.92	65.0	77.0	2.38	0.45	4.45	14.9	1.209	0.009	1.894	0.676
148	6.21	13.92	61.0	85.0	2.10	0.50	4.17	16.5	1.183	0.000	1.818	0.635
150	6.20	13.92	60.0	20.0	2.12	0.12	4.10	3.88	0.621	0.000	1.245	0.624
ave	5.96	13.92	94.09	35.0	3.24	0.21	6.44	6.635	0.953	0.006	1.94	0.98
std	0.18	0.00	44.60	32.59	1.47	0.19	3.05	6.20	0.99	0.004	0.97	0.46
VS undigested					6.84	VS digested			7.08			
Water of hydrolysis					0.79	Closure			1.19			

Table N3B. Fermentation data for Fermentation Train B

Day	ave pH	VS in (g)	liq out (mL)	solid out (g)	acid out (g)	VS solid out (g)	VS liq out (g)	VS residue (g)	Biotic CO_2 (g)	CH_4 (g)	Total gas out (g)	Abiotic CO_2 (g)
1	6.15	6.96	77.0	10.0	1.81	1.24	1.34	0.02	0.680	0.048	1.236	0.508
3	6.09	6.96	92.0	18.0	2.17	2.23	1.60	0.03	1.027	0.073	1.707	0.607
5	6.60	6.96	62.0	67.0	1.46	8.31	1.08	0.11	1.068	0.076	1.553	0.409
7	5.82	6.96	89.0	0.0	2.10	0.00	1.55	0	1.165	0.082	1.835	0.588
9	6.00	6.96	90.0	0.0	2.12	0.00	1.57	0	1.033	0.073	1.701	0.594
11	5.94	6.96	95.0	5.0	2.24	0.62	1.65	0.01	0.721	0.051	1.399	0.627
13	5.98	6.96	98.0	14.0	2.31	1.74	1.71	0.02	1.297	0.092	2.035	0.647
15	5.92	6.96	70.5	26.0	1.66	3.22	1.23	0.04	1.098	0.078	1.642	0.465
17	6.11	6.96	90.0	24.0	2.12	2.98	1.57	0.04	1.945	0.138	2.677	0.594
21	6.10	6.96	78.0	23.0	1.84	2.85	1.36	0.04	1.541	0.109	2.165	0.515
23	6.42	6.96	100.0	6.0	2.35	0.74	1.74	0.01	1.533	0.109	2.301	0.660
28	6.44	6.96	90.0	19.0	2.12	2.36	1.57	0.03	1.596	0.113	2.303	0.594
30	6.40	6.96	109.0	6.0	2.57	0.74	1.90	0.01	2.620	0.185	3.525	0.720
35	6.34	6.96	98.0	33.0	2.31	4.09	1.71	0.05	1.515	0.107	2.270	0.647
37	6.40	6.96	94.0	0.0	2.21	0.00	1.64	0	1.376	0.097	2.094	0.621
39	6.45	6.96	69.0	0.0	1.62	0.00	1.20	0	1.079	0.076	1.611	0.456
41	6.36	6.96	48.0	0.0	1.13	0.00	0.84	0	0.991	0.070	1.378	0.317
43	6.34	6.96	78.0	38.0	1.84	4.71	1.36	0.06	1.198	0.085	1.798	0.515
45	6.31	6.96	91.0	24.0	2.14	2.98	1.58	0.04	1.298	0.092	1.991	0.601
90	7.01	6.96	97.0	1.0	2.28	0.12	1.69	0	1.029	0.073	1.742	0.640
92	6.86	6.96	72.0	79.0	1.69	9.80	1.25	0.13	0.693	0.049	1.217	0.475
94	7.06	6.96	102.0	0.0	2.40	0.00	1.77	0	1.102	0.078	1.853	0.673
96	7.17	6.96	82.0	0.0	1.93	0.00	1.43	0	0.689	0.049	1.280	0.541
ave	6.36	6.96	85.72	17.1	2.02	2.12	1.49	0.027	1.230	0.087	1.88	0.57
std	0.37	0.00	14.49	21.30	0.34	2.64	0.25	0.03	0.44	0.031	0.52	0.10
VS undigested					2.15	VS digested			4.82			
Water of hydrolysis					0.54	Closure			0.93			

Table N3C. Fermentation data for Fermentation Train C

Day	ave pH	VS in (g)	liq out (mL)	solid out (g)	acid out (g)	VS solid out (g)	VS liq out (g)	VS residue (g)	Biotic CO_2 (g)	CH_4 (g)	Total gas out (g)	Abiotic CO_2 (g)
1	5.80	13.92	90.0	26.0	3.30	0.76	1.74	7.475	0.488	0.022	1.441	0.932
3	5.66	13.92	87.0	45.0	3.19	1.31	1.68	12.94	0.754	0.033	1.737	0.950
5	5.70	13.92	74.0	42.0	2.72	1.23	1.43	12.08	0.377	0.017	1.201	0.808
7	5.75	13.92	92.0	59.0	3.38	1.72	1.78	16.96	0.399	0.018	1.421	1.004
9	5.67	13.92	106.0	10.0	3.89	0.29	2.05	2.875	0.991	0.044	2.192	1.157
11	5.70	13.92	107.0	5.0	3.93	0.15	2.07	1.438	0.678	0.030	1.877	1.168
13	5.72	13.92	111.0	0.0	4.08	0.00	2.15	0	0.461	0.020	1.693	1.212
15	5.73	13.92	97.0	44.0	3.56	1.28	1.88	12.65	0.643	0.028	1.730	1.059
17	5.79	13.92	77.0	56.0	2.83	1.64	1.49	16.1	1.242	0.055	2.137	0.841
21	5.76	13.92	89.0	15.0	3.27	0.44	1.72	4.313	1.729	0.077	2.777	0.972
23	5.79	13.92	79.0	47.0	2.90	1.37	1.53	13.51	0.421	0.019	1.302	0.862
25	5.83	13.92	85.0	34.0	3.12	0.99	1.64	9.775	0.893	0.040	1.861	0.928
27	5.74	13.92	74.0	26.0	2.72	0.76	1.43	7.475	0.512	0.023	1.343	0.808
ave	5.74	13.92	89.85	31.5	3.30	0.92	1.74	9.045	0.738	0.033	1.75	0.98
std	0.05	0.00	12.50	19.45	0.46	0.57	0.24	5.59	0.40	0.017	0.44	0.14
VS undigested					9.96	VS digested			5.81			
Water of hydrolysis					0.65	Closure			1.08			

Table N3D. Fermentation data for Fermentation Train D

Day	ave pH	VS in (g)	liq out (mL)	solid out (g)	acid out (g)	VS solid out (g)	VS liq out (g)	VS residue (g)	Biotic CO_2 (g)	CH_4 (g)	Total gas out (g)	Abiotic CO_2 (g)
10	6.40	22.20	113.0	0.0	3.65	0.00	5.24	0	0.127	0.0006	1.159	1.031
12	5.99	22.20	160.0	0.0	5.34	0.00	7.42	0	0.619	0.0028	2.133	1.510
14	5.97	22.20	166.0	0.0	6.45	0.00	7.70	0	0.479	0.0022	2.303	1.822
16	5.46	22.20	110.0	0.0	4.31	0.00	5.10	0	0.591	0.0027	1.812	1.218
18	5.44	22.20	65.0	30.0	1.88	3.60	3.02	4.47	0.619	0.0028	1.153	0.531
20	6.26	22.20	109.0	42.0	4.33	5.04	5.06	6.25	0.507	0.0023	1.733	1.224
22	5.51	22.20	102.0	52.0	4.02	6.24	4.73	7.74	0.479	0.0022	1.616	1.135
24	5.56	22.20	92.0	73.0	3.65	8.76	4.27	10.9	0.451	0.0020	1.484	1.032
26	5.61	22.20	68.0	77.0	2.66	9.24	3.16	11.5	0.880	0.0040	1.634	0.750
28	5.71	22.20	82.0	40.0	3.37	4.80	3.80	5.95	0.272	0.0012	1.225	0.952
30	5.74	22.20	55.0	91.0	1.83	10.92	2.55	13.5	0.521	0.0024	1.040	0.516
31	5.64	22.20	61.0	80.0	2.46	9.60	2.83	11.9	0.380	0.0017	1.078	0.696
34	5.67	22.20	100.0	33.0	4.01	3.96	4.64	4.91	0.296	0.0013	1.431	1.134
36	5.63	22.20	64.0	104.0	2.63	12.48	2.97	15.5	0.366	0.0017	1.112	0.744
38	5.73	22.20	175.0	18.0	6.32	2.16	8.12	2.68	0.798	0.0036	2.588	1.786
40	5.75	22.20	170.0	0.0	7.21	0.00	7.89	0	0.435	0.0020	2.475	2.038
42	5.76	22.20	81.0	24.0	3.48	2.88	3.76	3.57	0.214	0.0010	1.198	0.983
44	5.78	22.20	51.0	131.0	2.18	15.72	2.37	19.5	0.521	0.0024	1.139	0.616
46	5.80	22.20	101.0	66.0	4.23	7.92	4.69	9.82	0.704	0.0032	1.904	1.196
48	5.82	22.20	68.0	47.0	2.92	5.64	3.16	7	0.732	0.0033	1.561	0.826
50	5.87	22.20	67.0	98.0	2.79	11.76	3.11	14.6	0.296	0.0013	1.086	0.789
52	5.78	22.20	80.0	87.0	3.42	10.44	3.71	12.9	0.532	0.0024	1.502	0.968
54	5.69	22.20	83.0	15.0	3.55	1.80	3.85	2.23	0.276	0.0013	1.281	1.003
60	5.87	22.20	68.0	67.0	2.98	8.04	3.16	9.97	0.056	0.0003	0.898	0.841
62	5.82	22.20	47.0	119.0	1.64	14.28	2.18	17.7	0.451	0.0020	0.917	0.465
67	5.77	22.20	57.0	0.0	2.45	0.00	2.64	0	0.237	0.0011	0.929	0.691
71	5.78	22.20	83.0	85.0	3.91	10.20	3.85	12.7	0.225	0.0010	1.330	1.104
73	5.78	22.20	60.0	86.0	2.71	10.32	2.78	12.8	0.183	0.0008	0.949	0.765
75	5.79	22.20	94.0	24.0	3.34	2.88	4.36	3.57	0.225	0.0010	1.172	0.945
79	5.81	22.20	98.0	38.0	3.99	4.56	4.55	5.66	0.270	0.0012	1.400	1.128
81	5.61	22.20	87.0	29.0	4.06	3.48	4.04	4.32	0.527	0.0024	1.675	1.146
85	5.47	22.20	90.0	66.0	3.96	7.92	4.18	9.82	0.099	0.0004	1.219	1.120
87	5.50	22.20	87.0	78.0	3.99	9.36	4.04	11.6	0.310	0.0014	1.439	1.128
89	5.56	22.20	74.0	48.0	2.96	5.76	3.43	7.14	0.380	0.0017	1.218	0.836
91	5.58	22.20	52.0	76.0	2.26	9.12	2.41	11.3	0.197	0.0009	0.837	0.639
93	5.67	22.20	63.0	33.0	2.50	3.96	2.92	4.91	0.265	0.0012	0.973	0.707
95	5.67	22.20	150.0	41.0	5.44	4.92	6.96	6.1	0.120	0.0005	1.658	1.537
101	5.70	22.20	161.0	96.0	5.20	11.52	7.47	14.3	0.127	0.0006	1.598	1.471
103	5.73	22.20	88.0	37.0	2.80	4.44	4.08	5.51	0.211	0.0010	1.005	0.793
105	5.83	22.20	92.0	43.0	3.66	5.16	4.27	6.4	0.338	0.0015	1.374	1.035
111	5.84	22.20	101.0	43.0	3.45	5.16	4.69	6.4	0.011	0.0001	0.986	0.974
113	5.84	22.20	104.0	56.0	4.53	6.72	4.83	8.34	0.042	0.0002	1.323	1.281

116	5.80	22.20	79.0	56.0	3.08	6.72	3.67	8.34	0.449	0.0020	1.321	0.870
ave	5.74	22.20	92.05	51.8	3.62	6.22	4.27	7.716	0.368	0.0017	1.39	1.02
std	0.18	0.00	33.96	34.25	1.24	4.11	1.58	5.10	0.21	0.0010	0.42	0.35
VS undigested					13.94	VS digested			8.26			
Water of hydrolysis					0.92	Closure			0.96			

Table N3E. Fermentation data for Fermentation Train E

Day	ave pH	VS in (g)	liq out (mL)	solid out (g)	acid out (g)	VS solid out (g)	VS liq out (g)	VS residue (g)	Biotic CO_2 (g)	CH_4 (g)	Total gas out (g)	Abiotic CO_2 (g)
10	6.34	11.10	130.0	0.0	2.81	0.00	4.20	0	0.227	0.0009	1.021	0.793
12	5.62	11.10	110.0	0.0	3.26	0.00	3.55	0	0.507	0.0020	1.431	0.922
14	5.82	11.10	115.0	0.0	3.90	0.00	3.71	0	0.437	0.0017	1.541	1.102
16	5.53	11.10	132.0	0.0	4.58	0.00	4.26	0	0.271	0.0011	1.567	1.295
18	5.64	11.10	114.0	0.0	3.99	0.00	3.68	0	0.262	0.0010	1.391	1.128
20	6.28	11.10	116.0	0.0	3.86	0.00	3.75	0	0.350	0.0014	1.442	1.091
22	5.69	11.10	95.0	0.0	3.20	0.00	3.07	0	0.647	0.0025	1.554	0.905
24	5.71	11.10	117.0	0.0	3.71	0.00	3.78	0	0.901	0.0035	1.953	1.049
26	5.71	11.10	140.0	0.0	4.46	0.00	4.52	0	0.402	0.0016	1.664	1.260
28	6.03	11.10	110.0	0.0	3.62	0.00	3.55	0	0.182	0.0007	1.205	1.023
30	6.03	11.10	74.0	0.0	2.36	0.00	2.39	0	0.577	0.0023	1.246	0.667
32	5.82	11.10	86.0	0.0	2.80	0.00	2.78	0	0.831	0.0033	1.625	0.791
34	5.84	11.10	76.0	0.0	2.57	0.00	2.45	0	0.939	0.0037	1.668	0.725
36	5.96	11.10	90.0	0.0	2.97	0.00	2.91	0	0.262	0.0010	1.102	0.839
38	5.75	11.10	77.0	0.0	2.72	0.00	2.49	0	0.516	0.0020	1.285	0.767
40	5.72	11.10	98.0	0.0	3.46	0.00	3.17	0	0.705	0.0028	1.684	0.977
42	5.88	11.10	94.0	0.0	3.35	0.00	3.04	0	0.234	0.0009	1.181	0.946
44	5.92	11.10	77.0	29.0	2.62	3.39	2.49	6.8672	0.367	0.0014	1.110	0.741
46	5.95	11.10	89.0	25.0	2.86	2.93	2.87	5.92	0.927	0.0036	1.738	0.807
48	5.90	11.10	81.0	30.0	2.66	3.51	2.62	7.104	1.049	0.0041	1.804	0.751
50	5.92	11.10	73.0	58.0	2.49	6.79	2.36	13.734	0.175	0.0007	0.879	0.703
52	5.92	11.10	81.0	38.0	2.41	4.45	2.62	8.9984	1.125	0.0044	1.811	0.682
54	5.66	11.10	162.0	0.0	4.82	0.00	5.23	0	0.213	0.0008	1.576	1.362
56	5.86	11.10	108.0	36.0	3.41	4.21	3.49	8.5248	0.418	0.0016	1.382	0.963
60	5.88	11.10	116.0	23.0	3.88	2.69	3.75	5.4464	0.105	0.0004	1.202	1.097
62	5.85	11.10	76.0	50.0	3.00	5.85	2.45	11.84	0.105	0.0004	0.953	0.847
64	5.84	11.10	82.0	12.0	2.85	1.40	2.65	2.8416	0.437	0.0017	1.244	0.805
68	5.82	11.10	91.0	9.0	3.34	1.05	2.94	2.1312	0.292	0.0011	1.236	0.943
72	5.79	11.10	97.0	59.0	3.69	6.90	3.13	13.971	0.297	0.0012	1.341	1.042
74	5.83	11.10	88.0	28.0	3.13	3.28	2.84	6.6304	0.087	0.0003	0.973	0.885
76	5.84	11.10	85.0	38.0	2.95	4.45	2.75	8.9984	0.122	0.0005	0.955	0.832
80	5.86	11.10	92.0	33.0	3.19	3.86	2.97	7.8144	0.486	0.0019	1.390	0.901
82	5.60	11.10	90.0	19.0	2.98	2.22	2.91	4.4992	0.138	0.0005	0.980	0.842
86	5.61	11.10	88.0	59.0	2.90	6.90	2.84	13.971	0.591	0.0023	1.413	0.820
88	5.62	11.10	103.0	12.0	3.39	1.40	3.33	2.8416	0.551	0.0022	1.512	0.959

90	5.64	11.10	100.0	29.0	2.99	3.39	3.23	6.8672	0.638	0.0025	1.487	0.846
92	5.68	11.10	74.0	72.0	2.36	8.42	2.39	17.05	0.262	0.0010	0.929	0.666
94	5.70	11.10	120.0	0.0	3.78	0.00	3.88	0	0.651	0.0025	1.722	1.069
96	5.68	11.10	106.0	35.0	3.49	4.10	3.42	8.288	0.112	0.0004	1.098	0.986
100	5.69	11.10	83.0	34.0	2.22	3.98	2.68	8.0512	0.175	0.0007	0.803	0.628
102	5.69	11.10	84.0	51.0	2.11	5.97	2.71	12.077	0.157	0.0006	0.753	0.595
104	5.69	11.10	134.0	13.0	4.27	1.52	4.33	3.0784	0.157	0.0006	1.364	1.206
106	5.78	11.10	100.0	28.0	2.80	3.28	3.23	6.6304	0.192	0.0008	0.985	0.792
114	5.78	11.10	77.0	35.0	1.83	4.10	2.49	8.288	0.017	0.0001	0.536	0.518
ave	5.80	11.10	98.43	19.4	3.18	2.27	3.18	4.601	0.411	0.0016	1.31	0.90
std	0.16	0.00	20.46	21.06	0.67	2.46	0.66	4.99	0.29	0.0011	0.32	0.19
VS undigested					6.87	VS digested			6.77			
Water of hydrolysis					0.75	Closure			1.15			

Table N3F. Fermentation data for Fermentation Train F

Day	ave pH	VS in (g)	liq out (mL)	solid out (g)	acid out (g)	VS solid out (g)	VS liq out (g)	VS residue (g)	Biotic CO_2 (g)	CH_4 (g)	Total gas out (g)	Abiotic CO_2 (g)
56	5.74	18.10	51.0	74.0	1.93	10.32	2.07	11.04	0.234	0.00078	0.780	0.546
60	5.62	18.10	79.0	31.0	3.11	4.32	3.21	4.625	0.522	0.00173	1.403	0.879
62	5.82	18.10	80.0	42.0	2.69	5.86	3.25	6.266	0.450	0.00149	1.211	0.759
64	5.53	18.10	49.0	80.0	2.02	11.16	1.99	11.94	0.279	0.00093	0.851	0.571
68	5.64	18.10	76.0	24.0	3.29	3.35	3.09	3.581	0.270	0.00090	1.201	0.930
72	6.28	18.10	87.0	1.0	3.90	0.14	3.53	0.149	0.360	0.00120	1.465	1.103
74	5.69	18.10	104.0	90.0	4.56	12.56	4.22	13.43	0.666	0.00221	1.958	1.289
76	5.71	18.10	96.0	65.0	4.04	9.07	3.90	9.698	0.928	0.00308	2.073	1.143
80	5.71	18.10	100.0	60.0	4.48	8.37	4.06	8.952	0.414	0.00137	1.681	1.266
82	6.03	18.10	71.0	32.0	3.11	4.46	2.88	4.774	0.187	0.00062	1.068	0.880
86	6.03	18.10	68.0	75.0	2.99	10.46	2.76	11.19	0.594	0.00197	1.442	0.846
88	5.82	18.10	102.0	43.0	4.55	6.00	4.14	6.416	0.855	0.00284	2.143	1.285
90	5.84	18.10	89.0	69.0	3.65	9.63	3.61	10.29	0.967	0.00321	2.002	1.032
92	5.96	18.10	76.0	46.0	3.20	6.42	3.09	6.863	0.270	0.00090	1.175	0.904
94	5.75	18.10	101.0	26.0	4.00	3.63	4.10	3.879	0.531	0.00176	1.663	1.130
96	5.72	18.10	101.0	47.0	4.23	6.56	4.10	7.012	0.726	0.00241	1.922	1.194
100	5.88	18.10	87.0	56.0	3.69	7.81	3.53	8.355	0.241	0.00080	1.285	1.043
102	5.92	18.10	71.0	85.0	2.97	11.86	2.88	12.68	0.378	0.00125	1.218	0.838
104	5.95	18.10	88.0	37.0	3.61	5.16	3.57	5.52	0.955	0.00317	1.977	1.019
106	5.90	18.10	92.0	43.0	3.45	6.00	3.74	6.416	1.081	0.00359	2.060	0.976
114	5.92	18.10	112.0	21.0	3.88	2.93	4.55	3.133	0.180	0.00060	1.276	1.096
117	5.92	18.10	102.0	46.0	3.82	6.42	4.14	6.863	1.158	0.00384	2.242	1.080
ave	5.83	18.10	85.55	49.7	3.51	6.93	3.47	7.413	0.557	0.00185	1.55	0.99
std	0.17	0.00	16.84	23.09	0.72	3.22	0.68	3.45	0.31	0.00104	0.44	0.20
VS undigested					14.34	VS digested			7.54			
Water of hydrolysis					0.84	Closure			1.155			

Table N3G. Acid production for Fermentation Train A

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)
2	3.680	2.290	2.220	0.420	0.300	0.070	8.980
4	4.000	2.620	2.720	0.580	0.400	0.080	10.400
7	8.030	4.630	4.650	2.070	1.380	0.160	20.920
8	8.420	4.640	4.650	2.440	1.870	0.220	22.240
12	13.970	6.700	9.070	4.100	2.850	0.460	37.150
14	13.500	5.500	8.910	3.950	2.540	0.460	34.860
16	13.700	5.200	8.450	3.700	2.510	0.460	34.020
18	14.460	4.480	8.110	3.720	2.880	0.520	34.170
20	14.920	4.370	8.640	3.770	3.130	0.570	35.400
22	14.700	3.800	8.740	3.650	3.460	0.650	35.000
24	14.360	3.430	10.130	3.700	4.240	0.770	36.630
26	14.400	3.390	9.990	3.640	4.160	0.760	36.340
28	13.650	3.240	9.220	3.540	4.120	0.740	34.510
30	13.890	2.870	8.400	3.320	4.330	0.710	33.520
32	15.230	2.750	8.980	3.450	5.030	0.830	36.270
34	14.950	2.880	8.050	3.030	4.310	0.730	33.950
36	15.810	2.700	7.790	2.950	4.420	0.680	34.350
40	16.890	2.160	8.130	3.130	5.390	0.780	36.480
50	14.730	1.850	9.860	3.010	6.110	0.940	36.500
52	14.960	2.190	9.040	2.830	5.560	0.850	35.430
54	14.570	2.100	8.530	2.670	5.580	0.820	34.270
60	14.840	2.080	8.560	2.550	6.080	0.910	35.020
63	15.370	2.020	8.460	2.530	6.120	0.900	35.400
65	15.480	2.000	8.840	2.460	5.970	0.820	35.570
67	14.740	2.390	8.870	2.380	5.380	0.760	34.520
69	15.240	2.090	8.560	2.390	5.610	0.760	34.650
73	14.580	2.030	9.170	2.410	5.670	0.770	34.630
75	13.730	2.430	8.390	2.200	4.730	0.660	32.140
77	14.410	2.500	7.730	2.450	5.150	0.730	32.970
79	14.500	2.720	7.510	2.320	4.610	0.670	32.330
81	15.460	2.710	7.240	2.530	4.710	0.680	33.330
83	16.170	2.660	7.530	2.610	4.860	0.710	34.540
85	15.774	2.674	7.581	2.596	4.852	0.753	34.231
91	15.131	2.628	7.135	2.421	4.367	0.709	32.391
95	15.970	2.701	8.697	2.586	4.797	0.762	35.514
101	16.012	2.230	9.582	2.681	5.374	0.767	36.646
105	17.450	2.736	9.902	2.861	5.352	0.720	39.022
136	17.369	2.814	10.651	2.975	5.604	0.746	40.160
138	15.051	2.736	9.707	2.888	5.314	0.652	36.349
140	15.378	3.051	9.177	2.879	4.965	0.611	36.061
142	14.623	2.745	8.740	2.736	4.853	0.654	34.351
144	15.124	3.133	8.603	2.843	4.749	0.626	35.077

146	16.136	3.500	8.602	3.027	4.714	0.623	36.602
148	14.767	3.600	8.354	2.924	4.194	0.566	34.405
150	14.696	3.282	9.319	3.073	4.425	0.592	35.387
ave	15.041	3.002	8.706	2.963	4.709	0.704	35.126
std dev.	0.923	1.015	0.800	0.506	0.950	0.116	1.612
ratio	42.821	8.546	24.786	8.435	13.406	2.006	100.0
std dev.	2.212	2.845	1.552	1.370	2.650	0.327	0.000

Table N3H. Acid production for Fermentation Train B

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)
1	10.667	4.899	5.856	2.275	1.873	0.545	26.115
7	11.241	5.215	7.644	2.229	1.869	0.389	28.587
9	12.235	5.605	8.618	2.350	1.934	0.340	31.082
11	11.709	5.296	7.917	2.173	1.810	0.332	29.236
13	9.161	4.860	7.548	2.070	1.776	0.282	25.698
17	9.338	5.273	8.193	2.215	2.011	0.315	27.346
23	6.829	5.702	7.349	2.321	1.892	0.337	24.430
30	7.460	6.406	6.065	2.202	1.351	0.246	23.730
35	9.253	5.779	5.495	2.497	1.363	0.160	24.546
37	8.840	5.321	5.244	2.135	1.203	0.151	22.893
39	9.253	5.779	5.495	2.497	1.363	0.160	24.546
43	8.608	5.777	5.206	2.260	1.244	0.157	23.251
90	6.399	4.195	7.322	2.277	1.251	0.135	21.58
92	7.411	5.036	6.415	1.882	1.125	0.12	21.99
94	8.425	4.724	6.092	1.869	1.22	0.128	22.458
96	8.558	4.722	6.306	1.892	1.345	0.127	22.95
ave	9.087	5.287	6.673	2.196	1.539	0.245	25.027
std dev.	1.690	0.547	1.119	0.193	0.321	0.123	2.784
ratio	36.122	21.301	26.670	8.844	6.109	0.954	100.000
std dev.	3.780	2.781	3.458	0.990	0.813	0.423	0.000

Table N3I. Acid production for Fermentation Train C

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)
1	16.753	1.391	10.305	1.962	6.204	0.224	36.840
3	17.132	1.417	10.026	1.850	6.102	0.206	36.733
5	18.211	1.456	9.976	1.806	6.044	0.210	37.703
7	17.665	1.376	9.868	1.744	5.879	0.209	36.741
9	17.539	1.338	9.462	1.633	5.605	0.207	35.785
11	18.533	1.276	10.113	1.691	5.927	0.214	37.754
13	18.488	1.266	10.033	1.678	5.889	0.214	37.569
15	18.272	1.292	10.260	1.639	5.843	0.228	37.534
17	17.235	1.321	9.788	1.512	5.420	0.205	35.481
21	18.447	1.294	10.169	1.517	5.393	0.201	37.021
23	17.771	1.245	9.931	1.404	5.482	0.200	36.032
25	17.514	1.311	9.698	1.373	5.141	0.173	35.210
27	18.399	1.364	10.173	1.453	5.386	0.174	36.949
ave	17.843	1.334	9.985	1.636	5.716	0.205	36.719
std. dev	0.591	0.063	0.238	0.179	0.330	0.016	0.853
ratio	48.596	3.635	27.196	4.451	15.563	0.558	100.0
std. dev	1.275	0.180	0.488	0.445	0.723	0.039	0.000

Table N3J. Acid production for Fermentation Train D

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)
10	13.020	4.803	8.607	2.657	3.138	0.072	32.297
12	13.759	3.267	9.548	2.705	3.974	0.147	33.401
14	16.252	3.048	11.566	3.079	4.694	0.193	38.832
16	15.537	2.501	12.195	3.354	5.388	0.215	39.191
18	10.023	2.468	10.247	2.328	3.658	0.192	28.916
20	17.134	2.106	11.362	3.045	5.826	0.244	39.718
22	17.619	2.002	10.829	2.849	5.839	0.249	39.387
24	18.532	1.860	10.393	2.684	5.954	0.252	39.676
26	18.487	1.618	9.967	2.521	6.200	0.252	39.045
28	19.831	1.551	10.446	2.474	6.524	0.263	41.089
30	14.690	1.561	9.049	2.035	5.630	0.258	33.225
31	20.294	1.443	9.696	2.238	6.448	0.261	40.380
34	20.342	1.328	9.587	2.161	6.449	0.250	40.117
36	21.195	1.230	9.821	2.146	6.533	0.223	41.148
38	18.420	0.887	8.435	1.786	6.340	0.240	36.108
40	22.138	1.208	10.233	1.715	6.858	0.258	42.409
42	22.514	1.221	10.317	1.730	6.917	0.263	42.960
44	22.422	1.348	10.554	1.700	6.457	0.239	42.720
46	22.314	1.228	10.297	1.637	6.196	0.240	41.911
48	23.319	1.211	10.325	1.600	6.289	0.223	42.968
50	22.608	1.191	10.038	1.539	6.067	0.201	41.644
52	22.862	1.193	10.250	1.566	6.758	0.178	42.807
54	22.834	1.176	10.230	1.569	6.762	0.198	42.771
60	23.515	1.368	10.514	1.752	6.429	0.191	43.769
62	17.162	1.489	9.621	1.321	5.210	0.187	34.990
67	23.617	1.213	10.058	1.477	6.383	0.159	42.906
71	22.428	3.137	12.889	1.614	6.800	0.196	47.063
73	21.499	4.498	11.731	1.466	5.770	0.169	45.132
75	17.170	4.139	8.750	1.197	4.094	0.231	35.579
79	19.878	5.056	9.710	1.470	4.500	0.121	40.736
81	22.192	6.392	11.266	1.788	4.826	0.148	46.611
85	20.956	6.014	10.564	1.829	4.480	0.184	44.028
87	21.062	6.431	11.760	1.970	4.521	0.128	45.872
89	19.386	4.888	9.968	1.701	3.916	0.108	39.968
91	21.069	5.285	10.439	2.118	4.459	0.121	43.492
93	19.563	4.775	9.252	2.033	3.974	0.117	39.715
95	16.953	3.712	9.931	1.628	3.908	0.131	36.263
101	15.032	3.290	8.980	1.445	3.463	0.119	32.329
103	15.158	3.779	8.093	1.608	3.153	0.082	31.873
105	19.155	4.995	9.260	2.124	4.141	0.130	39.805
111	17.656	3.514	7.376	1.885	3.581	0.124	34.137
113	21.515	3.989	10.398	2.611	4.887	0.174	43.574
116	20.135	3.640	8.495	2.408	4.149	0.152	38.978

119	20.567	3.820	9.931	2.485	4.660	0.167	41.630
ave	19.359	2.884	10.068	2.024	5.277	0.188	39.799
std. dev	3.178	1.659	1.082	0.524	1.183	0.055	4.271
ratio	48.405	7.316	25.417	5.166	13.222	0.473	100.000
std. dev	4.357	4.095	2.553	1.547	2.476	0.142	0.000

Table N3K. Acid production for Fermentation Train RSE

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)
10	9.001	2.911	5.699	1.515	2.455	0.000	21.582
12	11.840	3.311	9.005	2.270	3.122	0.098	29.646
14	12.914	3.265	10.635	2.978	3.972	0.145	33.909
16	12.754	3.007	11.608	2.938	4.253	0.154	34.715
18	12.881	2.696	12.272	2.751	4.235	0.165	34.998
20	12.262	2.276	11.920	2.432	4.225	0.166	33.282
22	12.485	2.032	12.154	2.286	4.559	0.174	33.690
24	11.704	1.694	11.185	2.064	4.894	0.178	31.719
26	11.109	1.432	11.829	1.966	5.320	0.183	31.839
28	11.895	1.144	12.022	1.857	5.792	0.183	32.893
30	12.937	1.070	10.280	1.620	5.793	0.183	31.884
32	14.650	1.035	9.909	1.421	5.345	0.175	32.534
34	15.377	1.048	10.012	1.512	5.639	0.180	33.768
36	15.500	1.028	9.438	1.456	5.384	0.175	32.980
38	16.848	0.999	10.174	1.514	5.548	0.179	35.262
40	16.670	1.048	9.998	1.600	5.771	0.180	35.267
42	16.760	1.064	10.104	1.621	5.876	0.187	35.612
44	16.367	1.133	9.107	1.590	5.651	0.194	34.042
46	16.052	1.204	7.990	1.518	5.138	0.187	32.090
48	16.580	1.232	8.151	1.546	5.094	0.184	32.785
50	17.414	1.300	8.519	1.589	5.083	0.188	34.092
52	12.766	1.733	9.594	1.374	4.144	0.187	29.799
54	12.735	1.733	9.587	1.405	4.152	0.140	29.753
56	13.455	1.726	9.853	1.666	4.684	0.160	31.544
60	13.675	1.702	11.077	1.833	4.992	0.173	33.453
62	19.456	1.414	11.429	1.470	5.510	0.163	39.443
64	14.905	1.927	10.663	1.913	5.151	0.197	34.756
68	14.574	1.951	12.432	2.077	5.419	0.199	36.652
72	16.053	1.850	11.743	2.177	5.977	0.216	38.015
74	16.212	1.724	9.790	2.041	5.605	0.214	35.585
76	16.908	1.715	8.687	1.914	5.226	0.202	34.652
78	17.727	1.799	8.306	1.795	4.830	0.212	34.668
80	16.563	1.675	8.634	1.695	4.306	0.209	33.083
82	16.413	1.659	8.657	1.692	4.329	0.214	32.964
86	15.292	1.643	9.667	1.769	4.363	0.202	32.938
88	14.435	1.573	8.223	1.638	3.886	0.186	29.940

90	15.410	1.664	8.726	1.744	4.111	0.186	31.841
92	15.282	1.621	8.784	1.665	3.978	0.188	31.517
94	14.713	2.435	9.608	1.774	4.099	0.272	32.900
96	12.079	1.995	7.863	1.371	3.302	0.153	26.762
100	10.693	1.576	7.423	1.472	3.744	0.165	25.074
102	14.527	1.819	8.738	1.964	4.608	0.191	31.847
104	13.336	1.706	7.211	1.590	4.014	0.170	28.027
106	10.732	1.673	6.790	1.250	3.231	0.146	23.821
114	12.146	1.858	11.306	1.865	4.377	0.176	31.728
117	13.189	1.602	10.456	1.993	5.367	0.196	32.803
ave	14.289	1.733	9.723	1.808	4.708	0.178	32.438
std. dev	2.232	0.576	1.583	0.392	0.831	0.037	3.352
ratio	44.013	5.455	29.938	5.590	14.458	0.546	100.000
std. dev	4.719	2.147	3.538	1.097	1.757	0.112	0.000

Table N3L. Acid production for Fermentation Train F

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)
56	18.51728	1.415994	10.87937	1.467666	5.399431	0.17087	37.85061
60	19.54508	1.377296	11.44713	1.435573	5.393118	0.178031	39.37623
62	13.94173	1.830801	10.70894	1.870221	5.023831	0.189551	33.56508
64	20.51024	1.545061	11.76851	1.530952	5.741883	0.16103	41.25768
68	21.62085	1.542531	12.2166	1.542445	6.209652	0.17177	43.30385
72	22.09143	2.402146	12.27042	1.623341	6.306215	0.177381	44.87094
74	22.00506	2.513325	11.48952	1.648123	6.041606	0.166431	43.86406
76	21.32842	3.138372	10.24946	1.744497	5.484806	0.167918	42.11348
78	22.84748	4.150926	10.30112	1.972505	5.354393	0.159697	44.78612
80	22.03107	5.012481	9.863909	1.957968	4.831017	0.155222	43.85167
82	22.04197	5.017644	9.887776	2.027434	4.871893	0.156446	44.00317
86	21.98974	5.1121	10.28556	2.190711	4.833088	0.162959	44.57416
88	21.04041	4.272037	9.073666	2.059501	4.426569	0.152076	41.02426
90	21.96143	4.280788	9.061064	2.179315	4.434829	0.158675	42.0761
92	21.05373	3.747914	8.418273	2.113606	4.106244	0.153704	39.59347
94	21.97187	4.254958	8.851775	2.261268	4.320758	0.174993	41.83562
96	22.33242	4.309775	9.008216	2.250456	4.356875	0.170479	42.42822
100	21.86682	4.113049	9.14863	2.290926	4.180909	0.165684	41.76602
102	21.58654	3.549433	8.9732	2.359243	4.336843	0.162813	40.96807
104	19.80727	2.838491	8.141743	2.188238	4.378461	0.174728	37.52893
106	19.02542	2.492317	7.082892	1.910856	3.948982	0.150263	34.61073
114	19.65891	3.875719	7.67384	2.02583	4.064849	0.164914	37.46406
117	18.20422	3.811536	7.135562	1.989288	3.644666	0.155592	34.94086
ave	20.73823	3.330639	9.736399	1.940868	4.856127	0.165271	40.76754
std. dev	1.973467	1.226864	1.527823	0.28411	0.760141	0.009775	3.365612
ratio	50.85399	8.131216	23.89341	4.792193	11.92068	0.408515	100
std. dev	2.561979	2.799772	3.410273	0.796977	1.646563	0.047463	0

Table N3M. Batch fermentation at 20g/L

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	9.7982	2.6376	1.8129	0.0831	0.3159	0	14.648	17.45784
1	9.42892	2.2867	2.7026	0.0811	0.3024	0	14.802	18.07605
2	11.3668	2.727	3.5788	0.1015	0.3664	0	18.14	22.3071
3	12.7732	2.8567	3.8157	0.2331	0.3673	0	20.046	24.55662
4	14.1179	3.1949	4.5313	0.1341	0.3969	0	22.375	27.47542
5	13.744	3.1123	4.4187	0.1294	0.3841	0	21.789	26.75638
6	13.6888	4.014	4.2919	0.2343	0.4809	0	22.71	28.16651
8	15.0959	4.2095	4.4778	0.4116	0.6491	0	24.844	30.85723
10	16.4257	4.3289	4.4927	0.4737	0.6891	0	26.41	32.58445
11	16.702	4.4049	4.5255	0.514	0.7142	0	26.861	33.15396
12	16.7387	4.3414	4.551	0.5189	0.7227	0	26.873	33.17085
13	16.6473	4.2779	4.5772	0.5393	0.743	0	26.785	33.11503
14	16.9806	4.4997	4.6756	0.5771	0.7622	0	27.495	34.0439
15	16.6237	4.2082	4.7319	0.5689	0.7723	0	26.905	33.37399

Table N3N. Batch fermentation at 40g/L

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	2.2284	0	0.5755	0	0.3283	0	3.1322	3.891766
1	3.44791	0.195	1.0236	0.0983	0.3748	0	5.1396	6.438107
2	4.0375	0.2242	1.2225	0.0948	0.3584	0	5.9373	7.368207
3	5.17677	0.3607	2.3673	0.1019	0.3664	0	8.3731	10.68549
4	6.15967	1.9945	2.8492	0.1172	0.3787	0	11.499	14.86622
5	6.14625	1.9856	2.8181	0.1164	0.3772	0	11.444	14.78253
6	6.52	2.2176	2.8513	0.238	0.5451	0	12.372	16.1229
7	6.22805	2.0274	2.5963	0.3062	0.6388	0	11.797	15.45007
8	6.9135	2.2276	2.7701	0.3912	0.7724	0	13.075	17.1564
9	7.09823	2.2477	2.7793	0.4236	0.8172	0	13.366	17.54036
10	7.77009	2.407	3.1507	0.5832	0.9983	0	14.909	19.75273
11	7.93984	2.2425	3.6444	1.115	1.7212	0.0927	16.756	23.24912
12	8.1354	2.0945	3.5661	1.3725	2.1508	0.1	17.419	24.4995
13	8.38364	2.0454	3.895	1.6943	2.6581	0.1245	18.801	26.95908
14	8.06265	1.9196	3.7819	1.6043	2.5267	0.122	18.017	25.81651
15	8.46881	1.9531	4.1066	1.6862	2.6079	0.1263	18.949	27.15867

Table N3O. Batch fermentation at 70g/L

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	1.9138	0	0.6734	0.0976	0.3855	0	3.0703	4.049141
1	2.7072	0	1.2217	0.1085	0.4053	0	4.4427	5.840553
2	3.30981	0.1992	1.391	0.1088	0.4002	0	5.409	7.005262
3	4.04465	0.4931	1.736	0.108	0.4016	0	6.7834	8.74817
4	4.76526	1.6807	2.1305	0.1443	0.4319	0	9.1527	11.96103
5	4.60834	1.6229	2.0649	0.1391	0.4239	0	8.8592	11.58354
6	5.37081	1.8744	2.2936	0.2283	0.588	0	10.355	13.60448
7	5.88162	1.945	2.3833	0.2769	0.6408	0	11.128	14.57116
8	6.55883	2.011	2.7314	0.692	1.0583	0	13.052	17.59533
9	5.41644	1.4492	2.0696	0.7462	1.1111	0	10.793	14.73818
10	7.08812	1.5203	2.5173	1.4922	2.1253	0	14.743	20.80351
11	7.04079	1.4795	2.6091	1.5394	2.2223	0.0858	14.977	21.33442
12	7.05661	1.4099	2.5483	1.4976	2.1732	0.105	14.791	21.00804
13	7.0214	1.3045	2.5771	1.4725	2.1305	0.0852	14.591	20.69227
14	6.99144	1.2326	2.6013	1.4815	2.1585	0.0876	14.553	20.68187
15	7.12094	1.1853	2.741	1.5262	2.2079	0.0834	14.865	21.16122

Table N3P. Batch fermentation at 100g/L

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	1.51956	0	0.5395	0	0.3165	0	2.3755	3.096388
1	2.00061	0.1223	0.7664	0	0.317	0	3.2062	4.13965
2	2.58527	0.1739	0.8168	0.0852	0.311	0	3.9722	5.034844
3	3.09535	0.2956	1.1023	0.0792	0.3032	0	4.8756	6.177466
4	4.05831	1.102	1.5844	0.1033	0.325	0	7.1729	9.200037
5	4.01897	1.0964	1.5694	0.1029	0.3295	0	7.1172	9.135933
6	4.2318	1.219	1.8352	0.1291	0.339	0	7.754	10.04603
7	4.6252	1.2496	2.0884	0.147	0.3617	0	8.472	10.99668
8	5.01629	1.1334	1.9581	0.3946	0.7213	0	9.2238	12.21922
9	5.32118	1.0488	1.9876	0.5062	0.8979	0	9.7616	13.03348
10	5.83592	0.9843	1.9943	0.639	1.122	0	10.576	14.18694
11	5.92687	0.9339	2.0493	0.6396	1.1307	0	10.68	14.31911
12	6.13431	0.9263	2.1762	0.7041	1.2345	0	11.175	15.07093
13	6.81453	0.9484	2.3973	0.7755	1.3448	0	12.281	16.52518
14	5.7363	0.7092	2.061	0.6753	1.2181	0	10.4	14.07851
15	6.56792	0.7467	2.3533	0.7431	1.3078	0	11.719	15.77827

Table N3Q. Batch fermentation at 100^{+}g/L

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	1.48023	0	0.6669	0.0962	0.3845	0	2.6278	3.599451
1	2.13419	0	0.8213	0.1084	0.4197	0	3.4835	4.613431
2	2.58699	0.1808	0.8997	0.1011	0.4128	0	4.1813	5.428876
3	2.98115	0.381	1.0504	0.1047	0.4081	0	4.9253	6.361961
4	3.85973	0.8011	1.4028	0.1203	0.431	0	6.615	8.516403
5	3.96363	0.8153	1.4283	0.1322	0.4431	0	6.7825	8.731876
6	4.3265	0.855	1.6308	0.1522	0.4686	0	7.4331	9.587999
7	4.3271	0.7903	1.6453	0.1646	0.5299	0	7.4572	9.672077
8	4.25208	0.8861	1.7321	0.1832	0.6743	0	7.7279	10.21582
9	4.83177	0.9125	1.8234	0.1923	0.6943	0	8.4544	11.0481
10	5.06256	0.9342	1.8452	0.2012	0.7123	0	8.7555	11.40135
11	5.17736	0.9543	1.9231	0.2131	0.8213	0	9.0893	11.92625
12	5.86041	0.9759	2.0123	0.2213	0.8324	0	9.9023	12.83121
13	6.29312	1.0013	2.1002	0.2412	0.9123	0	10.548	13.65384
14	6.03331	1.0023	2.1301	0.2532	0.9213	0	10.34	13.48795
15	5.85462	1.0024	2.2314	0.2531	0.9321	0	10.274	13.50424

Table N4A. Acid concentration in F1 (Train A)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	6.095627	0.523114	1.42405	0.104092	0.135672	0	8.282555	9.753063
3	7.469619	0.629087	2.294183	0.237582	0.428568	0	11.05904	13.62502
7	9.359959	0.775006	3.336918	0.424443	0.974291	0	14.87062	18.99058
11	10.11778	1.01847	2.986146	0.370232	0.821415	0	15.31404	19.07609
13	11.62756	1.578686	3.6989	0.41167	1.120414	0	18.43723	23.29704
17	10.35536	1.923297	4.924907	0.989686	2.839543	0.172853	21.20565	29.64969
21	10.15669	2.093306	5.047455	1.113948	3.462599	0.263588	22.13758	31.62834
25	10.99813	2.566162	5.579701	1.321882	3.947751	0.317705	24.73133	35.57117
29	10.49872	2.333825	5.18795	1.313302	4.205677	0.393245	23.93272	34.75651
31	10.75174	2.402308	5.277484	1.351128	4.378609	0.426069	24.58734	35.76221
34	11.49936	2.53309	5.568442	1.48258	5.024819	0.538764	26.64705	39.02942
38	9.925851	2.18257	5.00988	1.424102	4.936443	0.558357	24.0372	35.75205
42	10.25583	2.223511	5.57621	1.679691	5.73276	0.651018	26.11902	39.44785
46	10.1071	2.006901	6.482216	2.206514	7.660836	0.827996	29.29156	45.92577
50	10.89832	1.93806	6.727167	2.390701	8.261681	0.875261	31.09119	48.73805
54	11.84258	1.729883	7.260651	2.856887	9.29092	0.864001	33.84492	53.29607
58	8.031986	1.213329	6.945008	2.840377	10.49636	0.828615	30.35567	50.59712
62	8.622417	1.2145	7.866985	3.271792	12.23426	0.937381	34.14734	57.42534
67	7.629901	1.099818	7.655651	3.297134	12.50983	0.861461	33.0538	56.36113
70	6.109106	0.859431	6.374705	2.830926	11.29793	0.73997	28.21207	48.64439
75	8.722978	0.945497	6.406439	2.897448	12.51519	0.875726	32.36327	54.3825
79	5.616048	0.600082	5.524392	2.748782	13.27181	0.796971	28.55809	50.38721
83	5.023145	0.516718	5.174025	2.573804	12.49214	0.741191	26.52102	47.00605
87	6.516503	0.623157	5.428641	2.655749	12.85091	0.799332	28.87429	50.11344
91	5.130708	0.563837	5.294896	2.654336	12.97684	0.768156	27.38878	48.60375
96	5.034954	0.568721	5.161049	2.612756	13.10965	0.764974	27.25211	48.47498
100	5.813343	0.580259	5.849496	3.073391	14.04898	0.746562	30.11203	53.23089
104	5.778709	0.557835	5.435543	2.80131	13.88109	0.772926	29.22741	51.64779
108	6.753333	0.604516	5.43111	2.79766	14.20535	0.78116	30.57313	53.36449
112	2.739258	0.15601	2.617631	1.252366	11.89199	0.628944	19.2862	35.82952
116	4.86459	0.262553	3.020101	1.29031	12.21251	0.65008	22.30014	39.57719
121	5.129861	0.302323	3.227161	1.351289	12.13426	0.641486	22.78638	40.18819
125	9.36285	0.47787	3.36657	1.275893	11.28385	0.697645	26.46468	43.12986
129	3.192889	0.164287	2.414711	1.049049	10.53468	0.546474	17.90209	32.56856
131	3.898535	0.182606	2.449662	1.01355	10.40269	0.552434	18.49948	33.03229
133	4.23746	0.167834	2.640318	1.032673	10.92845	0.593044	19.59978	34.89015
137	4.149479	0.181304	2.53942	1.00573	10.58156	0.571091	19.02859	33.83108
141	4.26939	0.197999	2.548117	0.985953	10.3889	0.569689	18.96005	33.54978
145	4.368744	0.180499	2.605757	1.416307	9.603705	0.569433	18.74445	32.91965
149	2.904878	0.126789	1.543946	0.756984	5.681644	0.763515	11.77776	20.60983
153	4.808219	0.284766	3.316941	1.834327	12.79766	0.773598	23.81551	42.5823
157	3.452637	0.185941	2.347721	1.085973	8.303036	0.490836	15.86614	28.07253
161	5.155957	0.328745	3.228117	1.769228	12.74153	0.762662	23.98624	42.57646
165	3.023601	0.148736	1.85843	0.77204	6.306615	0.357237	12.46666	21.72782

169	3.126561	0.144326	1.834382	0.750326	6.112251	0.345362	12.31321	21.31351
177	4.124954	0.168275	1.968265	0.748019	5.902737	0.342647	13.2549	22.13093
181	3.969473	0.288889	1.851287	0.803099	6.339236	0.356579	13.60856	22.98685
185	4.181203	0.192359	1.974568	0.861042	6.725397	0.385705	14.32027	24.24638
189	4.069543	0.171174	1.539534	0.556177	5.036155	0.278792	11.65138	19.0466
193	4.261651	0.185716	1.574283	0.586595	5.28277	0.295088	12.1861	19.92341
197	4.474124	0.199778	1.58286	0.589159	5.246031	0.301856	12.39381	20.11435
201	4.557346	0.210675	1.66253	0.588181	5.246375	0.305839	12.57095	20.35661
205	4.893264	0.226183	1.549626	0.535288	4.691656	0.283911	12.17993	19.22425
209	4.265979	0.215235	1.408356	0.518375	4.790516	0.277158	11.47562	18.49743
213	4.145675	0.218251	1.361963	0.477831	4.337534	0.247452	10.78871	17.22142
217	3.796363	0.184696	1.828272	0.478846	4.225428	0.243774	10.75738	17.38141
221	3.188354	0.161283	2.143296	0.469948	4.266378	0.213065	10.44232	17.27746
225	3.243895	0.182414	2.125934	0.451858	4.101946	0.199646	10.30569	16.92889
229	2.936054	0.173287	2.223215	0.447168	4.110826	0.188075	10.07862	16.75781
233	2.798354	0.187026	2.159156	0.436452	4.163658	0.177454	9.9221	16.59582
237	1.817727	0.134887	0	0.258921	2.263392	0.181356	4.656283	7.590906
241	2.967178	0.205155	1.569146	0.325274	2.995503	0.151316	8.213573	13.09503
245	2.883523	0.21262	0	0.279328	2.532555	0.122477	6.030503	9.23519
249	3.160567	0.238938	0	0.271697	2.4881	0.118229	6.277531	9.433863
253	2.712518	0.219288	0.850329	0.179972	1.657012	0.079979	5.699097	8.427813
257	2.637044	0.237004	0.804234	0	1.534461	0	5.212742	7.525199
261	3.69406	0.344896	0.963926	0.372847	1.712609	0.085304	7.173642	10.27819
265	3.649757	0.314823	0.935538	0.302639	1.849169	0.0962	7.148126	10.31506
267	3.031182	0.156633	0.799507	0.328848	2.118941	0.107681	6.542792	9.873213
271	1.386097	0.141713	0.456643	0.115187	0.926028	0	3.025667	4.505426
275	0.866728	0	0.385128	0	0.466567	0	1.718424	2.490582
279	1.757722	0.185418	0.628314	0	0.289455	0.393743	3.254652	4.557678
283	0.920668	0.408354	0.302819	0	0.1304	0	1.762241	2.287956
289	0.942234	0	0.239209	0	0	0	1.181443	1.351101
293	1.67975	0.258155	0.308684	0	0.116841	0	2.36343	2.816302
297	3.409349	0.303719	0.611707	0.127035	0.113153	0	4.564963	5.364422
301	4.843709	0.435551	1.252534	0.383833	1.744287	0.134683	8.794598	12.24485

Table N4B. Acid concentration in F2 (Train A)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	4.765688	0.421586	1.106714	0	0.159529	0	6.453517	7.586705
3	8.18893	0.752758	2.230307	0.237855	0.358465	0	11.76831	14.26695
7	9.472551	1.058948	3.208363	0.415139	0.779062	0	14.93406	18.86512
11	10.14328	1.559328	3.139547	0.484482	0.977412	0	16.30405	20.673
13	12.06226	1.918745	3.028793	0.53813	1.284291	0.112145	18.94436	23.89921
17	15.33455	2.008101	2.242887	0.589236	1.839368	0.328006	22.34215	27.68119
21	15.87716	2.031401	2.311057	0.648865	2.121138	0.423422	23.41304	29.28054
25	16.61728	1.995149	2.293623	0.696696	2.524241	0.579908	24.70689	31.20963
29	15.68737	1.796316	2.119154	0.673051	2.658177	0.661961	23.59603	30.11108
31	17.68557	1.882055	2.227759	0.755191	3.371396	0.910234	26.83221	34.59674
34	17.24752	1.844336	2.364655	0.788355	3.543642	0.951393	26.73991	34.84873
38	16.92605	1.689601	2.734267	0.864412	4.136897	1.146106	27.49733	36.73899
42	15.6103	1.389074	2.953143	0.83037	4.343627	1.185383	26.3119	35.81789
46	16.43877	1.297854	3.657209	0.921182	5.236289	1.380426	28.93173	40.16875
50	16.29794	1.204348	4.221841	1.019173	5.86143	1.504837	30.10956	42.61319
54	17.14163	1.190892	4.19146	0.961823	5.535831	1.43464	30.45628	42.44868
58	21.75597	1.479245	4.352154	1.006721	5.639625	1.499406	35.73312	48.19343
62	21.59222	1.495397	4.25199	1.017123	5.356245	1.336706	35.04968	46.95773
67	22.80209	1.573517	4.176841	1.012153	5.179907	1.259769	36.00428	47.60789
70	25.11059	1.734947	4.413896	1.094403	5.361047	1.280846	38.99573	51.13067
75	26.03211	1.735692	4.431469	1.178764	5.619641	1.2843	40.28197	52.78845
79	26.95383	1.742488	4.446909	1.17952	5.558318	1.251382	41.13244	53.54917
83	26.53705	1.683955	4.355166	1.192466	5.487994	1.19706	40.45369	52.65237
87	27.56656	1.698331	4.299785	1.137137	5.139208	1.166809	41.00783	52.71421
91	26.88041	1.650487	4.390457	1.207485	5.463053	1.127563	40.71946	52.83331
96	27.17452	1.635742	4.285425	1.248548	5.586023	1.099511	41.02977	53.19919
100	27.41156	1.59722	4.52719	1.289933	5.630745	1.074789	41.53144	53.91171
104	28.33722	1.582904	4.596806	1.303865	5.730564	1.087449	42.63881	55.19771
108	28.25822	1.529597	4.428193	1.346563	5.982411	1.063986	42.60897	55.30699
112	25.70798	1.383828	4.258811	0.921476	5.734777	1.000271	39.00714	50.79275
116	26.34601	1.381054	4.332526	0.963834	6.160096	1.013999	40.19752	52.545
121	30.0116	1.503543	4.592046	0.944228	5.535411	1.053244	43.64007	55.58539
125	25.58302	1.261861	4.251547	0.920685	5.779119	0.967926	38.76416	50.50143
129	25.0627	1.205908	4.331943	0.909757	5.629902	0.952735	38.09295	49.67515
131	18.28438	0.772083	5.698367	1.033568	7.408623	0.821471	34.01849	48.23823
133	17.88701	0.717283	5.795579	1.094304	8.084442	0.825217	34.40383	49.45239
137	17.06208	0.658482	5.542684	1.073773	8.125446	0.837977	33.30044	48.18546
141	16.0164	0.588478	5.301025	1.061151	8.24724	0.809564	32.02386	46.79294
145	19.20027	0.817152	4.942125	1.353074	7.006904	0.907521	34.22705	47.89707
149	11.31662	0.450371	3.561553	0.993618	5.479299	0.587995	22.38945	32.57872
153	20.4273	0.88654	5.571555	1.394741	6.900044	0.961505	36.14169	50.27435
157	18.35386	0.807028	5.305887	1.335441	6.708884	0.920332	33.43143	47.0334
161	16.32915	0.714484	5.008157	1.297121	6.862774	0.862965	31.07465	44.48714
165	14.97138	0.663057	4.76357	1.208386	6.358961	0.816156	28.78151	41.32222

169	13.79254	0.602927	4.605984	1.162424	6.247784	0.780432	27.19209	39.39163
177	9.607557	0.528761	4.52744	1.136161	7.035866	0.605892	23.44168	36.1625
181	9.489354	0.37759	4.480406	1.0983	6.755114	0.598527	22.79929	35.07933
185	11.77749	0.522022	5.022961	1.194955	6.600213	0.719914	25.83755	38.63059
189	9.089459	0.34315	4.586614	1.107242	7.067362	0.588066	22.78189	35.45211
193	8.816814	0.333247	4.639459	1.107897	7.227079	0.571719	22.69621	35.55143
197	8.571474	0.309946	4.425219	1.074032	7.093624	0.53924	22.01353	34.49491
201	8.83475	0.307785	4.710907	1.137132	7.59427	0.559881	23.14473	36.44542
205	9.213264	0.396316	4.484673	1.090815	6.639227	0.639661	22.46396	34.67285
209	8.457554	0.353577	4.236406	0.998776	5.992135	0.563244	20.60169	31.74858
213	8.359447	0.273636	4.40377	1.033495	7.227715	0.486894	21.78496	34.2798
217	8.820988	0.271399	4.505667	1.068082	7.590045	0.51488	22.77106	35.79023
221	8.8495	0.29597	4.173638	0.99794	6.963154	0.494159	21.77436	33.80947
225	8.566323	0.252077	3.805418	0.914394	6.437725	0.437811	20.41375	31.46406
229	8.420433	0.24355	3.690584	0.862054	6.059821	0.405151	19.68159	30.1557
233	9.120777	0.288599	4.034439	0.899179	6.436444	0.420088	21.19953	32.39089
237	8.078356	0.250841	3.571281	0.813976	5.845903	0.350481	18.91084	28.96527
241	8.510855	0.295048	3.571318	0.832053	5.896248	0.369962	19.47548	29.64255
245	4.830597	0.189825	1.886929	0.443791	3.307548	0.220765	10.87946	16.50565
249	5.574414	0.218274	2.179017	0.505279	3.556224	0.236855	12.27006	18.45667
253	8.307888	0.308372	3.376977	0.752662	5.259463	0.331827	18.33719	27.57264
257	7.994722	0.268411	3.163167	0.740564	5.113021	0.32688	17.60677	26.50047
261	8.285374	0.342934	3.039779	0.733878	4.926871	0.324492	17.65333	26.28321
265	8.794992	0.310821	3.223104	0.797721	5.23833	0.342238	18.70721	27.86642
267	8.434395	0.208983	3.114979	0.833142	5.282738	0.350918	18.22515	27.35522
271	7.998531	0.201481	2.941553	0.795947	5.144823	0.33723	17.41956	26.2257
275	7.693433	0.190951	2.852322	0.808104	5.120816	0.34783	17.01346	25.74998
279	10.47854	0.224955	2.860123	0.801795	4.865466	0.364542	19.59542	28.09485
283	10.22593	0.193552	2.787768	0.79852	4.906439	0.384377	19.29658	27.79609
289	9.966383	0.232826	2.748109	0.873293	4.767849	0.386643	18.9751	27.38551
293	10.74974	0.165624	2.906246	0.873201	5.246773	0.419793	20.36137	29.40812
297	10.6441	0.216738	2.885056	0.870309	5.187457	0.418824	20.22248	29.20835
301	10.69248	0.309104	3.330199	0.895156	5.511664	0.470898	21.2095	30.98047

Table N4C. Acid concentration in F3 (Train A)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	6.003302	0.544371	1.392683	0.113446	0.156801	0	8.210603	9.698986
3	6.080076	0.554561	1.444888	0.109441	0.162306	0	8.351272	9.883091
7	9.020698	0.784635	3.072761	0.364636	0.687194	0	13.92992	17.50515
11	10.34294	1.288017	3.536157	0.522675	1.120899	0	16.81069	21.53434
13	10.51537	1.606351	3.391802	0.568855	1.297234	0.110913	17.49052	22.60956
17	12.00297	2.08567	3.786085	0.720335	1.73569	0.120019	20.45077	26.66944
21	13.92224	2.193356	3.246026	0.952791	2.801175	0.547521	23.66311	31.41097
25	14.39932	2.1439	3.044041	0.956114	3.18127	0.79133	24.51597	32.802
29	13.9205	1.972177	2.738393	0.895368	3.203669	0.899808	23.62992	31.72566
31	16.13294	2.263047	3.198302	1.141876	3.895515	1.107711	27.73939	37.49802
34	14.6313	1.859308	2.811611	1.008538	3.705793	1.191144	25.20769	34.29718
38	14.45443	1.683545	3.270249	1.087431	4.282925	1.420716	26.19929	36.50271
42	12.71437	1.387994	3.759238	1.11804	4.399102	1.346297	24.72505	35.31154
46	11.7091	1.154265	4.76433	1.301557	5.194289	1.396791	25.52034	37.79667
50	12.28936	1.113316	6.298169	1.622241	6.595132	1.475603	29.39382	44.62316
54	11.68671	1.003606	7.541273	1.889164	7.756781	1.352666	31.2302	48.63129
58	11.91066	0.992892	6.650602	1.707179	7.746393	1.450827	30.45855	47.16642
62	13.04412	1.051547	6.273099	1.616788	7.284805	1.361346	30.6317	46.41502
67	17.42927	1.293561	6.261321	1.619215	7.224283	1.3992	35.22685	51.0898
70	16.89037	1.234722	5.693964	1.474216	6.53141	1.281777	33.10646	47.5292
75	20.38798	1.340541	5.570767	1.426178	6.590534	1.282498	36.5985	51.00192
79	21.58833	1.372781	5.343278	1.365898	6.012176	1.242803	36.92527	50.46094
83	22.33747	1.355908	5.02762	1.276217	5.629658	1.4884	37.11527	50.22344
87	23.00906	1.352559	5.268758	1.410585	6.207991	1.149604	38.39856	52.01349
91	24.78503	1.405753	4.903032	1.20174	5.075677	1.080124	38.45135	50.34585
96	26.53967	1.477907	5.057668	1.239735	5.155959	1.090157	40.5611	52.72917
100	25.68909	1.366194	4.877066	1.166807	4.895341	0.989527	38.98403	50.51115
104	28.99838	1.466858	5.299555	1.278891	5.233077	1.052868	43.32963	55.73968
108	30.77559	1.543592	5.563231	1.304691	5.374131	1.051231	45.61246	58.41501
112	30.25203	1.506303	5.581283	0.926362	5.309017	1.01669	44.59168	56.93436
116	29.84999	1.511261	5.576494	0.944441	5.56145	1.032911	44.47655	57.12376
121	27.66015	1.361935	5.30795	0.932702	5.741856	1.002695	42.00728	54.54671
125	29.55299	1.471348	5.457151	0.909803	5.422893	1.003103	43.81729	56.14765
129	27.88426	1.356808	5.490411	0.930922	5.70684	0.953587	42.32283	54.89134
131	26.32906	1.266439	4.966956	0.868738	4.978003	0.86196	39.27115	50.48429
133	26.62708	1.25952	5.090362	0.912944	5.184383	0.86718	39.94147	51.50664
137	27.38214	1.31682	5.802827	0.937814	6.064788	0.946077	42.45046	55.60289
141	25.52763	1.189963	5.835603	0.945281	6.348979	0.907338	40.7548	54.14036
145	26.81777	1.235893	6.138492	1.346007	6.472028	0.919819	42.93001	57.06243
149	18.34187	0.827766	3.918919	0.931002	4.252889	0.643263	28.91571	38.21737
153	23.47225	1.103267	5.756437	1.23339	6.493875	0.902038	38.96126	52.66475
157	23.3341	1.073337	6.056517	1.272802	6.644453	0.907816	39.28902	53.3958
161	21.83912	1.017416	6.000371	1.248908	6.743093	0.890384	37.73929	51.84467
165	18.10813	0.803669	4.59685	1.037837	4.984457	0.697939	30.22888	40.94497

169	17.82474	0.767934	4.693122	1.043716	4.986633	0.677868	29.99401	40.74654
177	18.29934	0.640322	5.344185	1.124933	5.300176	0.628114	31.33707	42.8465
181	17.1698	0.78434	5.722664	1.097083	5.989928	0.72119	31.48501	44.1454
185	18.07196	0.802623	5.638072	1.117585	5.643663	0.724106	31.99801	44.25859
189	17.66428	0.630598	5.636169	1.131762	5.5902	0.648794	31.3018	43.3542
193	17.56323	0.561523	6.012871	1.19942	6.112157	0.651803	32.101	45.01448
197	17.88685	0.574447	6.142459	1.215412	6.232456	0.645215	32.69684	45.84305
201	18.44084	0.761493	5.327073	1.080452	5.159857	0.738887	31.5086	42.99838
205	17.3272	0.534718	6.210048	1.228463	6.669174	0.726154	32.69575	46.44868
209	17.04842	0.511562	6.345588	1.223011	6.574173	0.686766	32.38953	46.07445
213	16.63148	0.51034	5.93854	1.139782	5.969554	0.634554	30.82425	43.43522
217	17.34751	0.517519	6.790374	1.301386	7.214087	0.661114	33.832	48.55977
221	15.66196	0.464796	6.09098	1.187472	6.512117	0.595164	30.51249	43.78858
225	15.21165	0.387128	6.058404	1.195518	6.701936	0.597926	30.15257	43.58654
229	14.3247	0.369033	6.298061	1.176288	7.051552	0.577648	29.79728	43.72465
233	12.21883	0.303634	5.933659	1.05966	6.750631	0.507489	26.7739	39.90229
237	5.82833	0	2.442772	0.41817	2.966945	0.22765	11.88387	17.4437
241	8.103794	0.188781	3.463434	0.656481	4.153848	0.32299	16.88933	24.85323
245	5.528863	0.141074	2.445268	0.383804	3.001694	0.240915	11.74162	17.38388
249	10.92543	0.280617	4.988642	0.871612	5.745642	0.435573	23.24752	34.36435
253	10.94436	0.26533	5.239935	0.921324	6.150347	0.470589	23.99189	35.80024
257	8.047074	0.176131	3.647517	0.679898	4.484388	0.344729	17.37974	25.86949
261	11.90945	0.268563	4.948638	0.94556	5.830416	0.466986	24.36962	35.64978
265	12.8875	0.295533	5.0599	1.056638	5.84907	0.477683	25.62632	37.13138
267	10.66379	0.152385	4.248299	0.961789	4.966626	0.408045	21.40094	31.15643
271	12.62186	0.219001	4.953452	1.001252	5.945187	0.50818	25.24893	36.73521
275	11.57713	0.320023	4.400984	0.924969	5.392988	0.458384	23.07448	33.49193
279	15.11427	0.167982	4.464361	0.955927	5.3289	0.488082	26.51952	36.91591
283	16.22655	0.155219	4.736785	1.037576	5.699324	0.523978	28.37943	39.47776
289	12.87263	0.139899	3.795478	0.820743	4.455846	0.413388	22.49799	31.26107
293	15.59188	0	4.440721	1.034546	5.618635	0.513832	27.19961	37.92191
297	12.09146	0.100269	3.422151	0.840796	5.162104	0.639273	22.25605	31.78251
301	16.41837	0.336065	4.594606	1.006582	5.611513	0.574468	28.5416	39.55365

Table N4D. Acid concentration in F4 (Train A)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	5.248128	0.726834	1.312685	0.229619	0.205175	0	7.722442	9.388273
3	7.454516	0.729696	2.333661	0.29897	0.454269	0	11.27111	13.9908
7	8.576133	1.281478	2.679492	0.412212	0.692064	0	13.64138	17.19599
11	11.80691	2.07256	3.405438	0.567783	0.880466	0	18.73315	23.47909
13	11.80362	2.211132	2.981765	0.529115	1.069633	0.081208	18.67647	23.44572
17	12.79037	2.282928	2.881972	0.679089	1.362179	0.186819	20.18336	25.48946
21	13.79743	2.250421	2.811526	0.704078	1.963856	0.397514	21.92483	28.08701
25	14.32398	2.081672	2.736702	0.794452	2.864075	0.753725	23.55461	31.06528
29	14.8144	2.181908	2.853291	0.792423	2.892061	0.767416	24.3015	31.98146
31	16.33004	2.406907	3.194063	0.981571	3.307358	0.876178	27.09612	35.85972
34	13.89296	2.026031	2.873575	0.905923	3.180003	0.880538	23.75903	31.9341
38	13.68463	1.833941	3.218885	1.018035	3.723893	1.043415	24.5228	33.74001
42	13.91429	1.799544	3.823694	1.157756	4.199252	1.130349	26.02489	36.3952
46	12.95817	1.527466	4.655082	1.318148	5.015858	1.158074	26.6328	38.52888
50	12.62963	1.453852	5.291617	1.479421	5.810581	1.36019	28.02529	41.57911
54	11.84518	1.239392	6.092097	1.645283	6.877993	1.319846	29.01979	44.29414
58	13.37368	1.334444	6.456561	1.751205	7.663012	1.455489	32.03439	48.70649
62	13.64383	1.292798	6.28425	1.691436	7.430766	1.386767	31.72984	47.8777
67	14.99776	1.347872	6.014414	1.613894	7.08359	1.365393	32.42292	47.93649
70	15.9853	1.364089	5.832788	1.550567	6.586392	1.250581	32.56972	47.23541
75	18.82839	1.497655	5.873012	1.546829	6.565367	1.275116	35.58637	50.34231
79	19.45624	1.469742	5.684908	1.505649	6.320635	1.218337	35.65551	49.90001
83	21.58609	1.543799	5.671177	1.478576	6.077472	1.17025	37.52736	51.45255
87	22.09474	1.540933	5.520715	1.430208	5.881054	1.150563	37.61822	51.15842
91	23.20903	1.773014	5.51276	1.401043	5.635107	1.098778	38.62973	51.91093
96	24.87043	1.591453	5.689311	1.432578	5.719561	1.094316	40.39765	53.8425
100	25.72583	1.573349	5.746841	1.423985	5.664375	1.067776	41.20216	54.5821
104	27.38199	1.586328	5.998278	1.460989	5.80844	1.076905	43.31293	57.07616
108	27.24148	1.550668	6.13005	1.464718	5.843247	1.05143	43.2816	57.13301
112	27.86538	1.562954	6.232402	1.06276	5.868194	1.036336	43.62802	57.19892
116	27.49196	1.518427	6.386487	1.066997	5.875041	1.022592	43.36151	57.01696
121	26.55402	1.448054	6.185387	1.100276	6.28985	1.000002	42.57759	56.50828
125	28.36426	1.530901	6.317794	1.051954	5.594252	1.006709	43.86587	57.14545
129	27.50699	1.468725	5.9187	1.003273	5.322425	0.968391	42.18851	54.77824
131	28.23308	1.480105	6.009484	1.003322	5.340974	0.968591	43.03556	55.71492
133	29.27407	1.514481	6.002547	1.027645	5.333667	0.971393	44.12381	56.83131
137	29.51694	1.504	5.965084	1.030264	5.424801	0.96627	44.40736	57.17807
141	29.60565	1.484565	5.851018	1.006365	5.255453	0.972844	44.1759	56.66269
145	29.2273	1.434875	5.759315	1.352544	5.139326	0.948084	43.86144	56.42459
149	28.95051	1.40281	5.716998	1.32292	4.962517	0.921636	43.27739	55.54901
153	28.12595	1.355448	5.592383	1.283493	4.812797	0.916691	42.08677	54.04761
157	26.70305	1.271497	5.362807	1.24656	4.728475	0.881634	40.19402	51.79047
161	26.95802	1.27766	5.340723	1.252798	4.929628	0.919465	40.6783	52.5282
165	26.369	1.26043	5.166737	1.204163	4.687218	0.900555	39.5881	50.9809

169	24.35738	1.157218	4.919479	1.140146	4.485676	0.865923	36.92583	47.78341
177	21.40104	0.824324	5.767169	1.158586	4.664542	0.65182	34.46748	45.73388
181	21.22989	1.032609	4.747546	1.035919	4.047574	0.758645	32.85219	42.84107
185	22.01451	0.868908	5.721754	1.149314	4.646853	0.663513	35.06485	46.30499
189	21.79513	0.876461	5.638732	1.143153	4.624376	0.67438	34.75223	45.92003
193	22.8526	0.841369	5.935868	1.184976	4.847969	0.659361	36.32215	47.94551
197	20.5386	0.934029	5.101658	1.059404	4.199547	0.719925	32.55317	42.88798
201	20.16786	0.932649	5.060302	1.054677	4.155469	0.74738	32.11834	42.40434
205	20.62046	0.871782	5.37255	1.096279	4.46634	0.721872	33.14928	43.97091
209	22.36312	0.752745	6.125582	1.227924	5.099111	0.664908	36.23339	48.2678
213	21.13227	0.679544	5.779159	1.15377	4.794494	0.608766	34.148	45.44515
217	20.29924	0.613567	5.467788	1.090275	4.508752	0.571341	32.55096	43.19125
221	21.6297	0.673387	5.912347	1.173236	5.011385	0.624035	35.02409	46.68133
225	19.2422	0.566238	5.204954	1.041425	4.348056	0.53034	30.93321	41.10155
229	19.68423	0.576761	5.289128	1.060716	4.613059	0.576502	31.8004	42.38943
233	19.74734	0.565423	5.748058	1.1196	5.003728	0.564832	32.74898	44.11543
237	13.43027	0.343613	3.974433	0.773034	3.468596	0.379835	22.36978	30.20456
241	11.26091	0.297233	3.313259	0.648089	2.958068	0.333078	18.81064	25.44079
245	18.12855	0.486856	5.344036	1.020728	4.566404	0.492033	30.03861	40.43999
249	10.38755	0.29087	3.165556	0.588915	2.656721	0.305541	17.39515	23.50805
253	16.92583	0.360186	5.059924	0.945577	4.317678	0.459627	28.06882	37.84177
257	7.788989	0.139778	3.288675	0.518485	3.446354	0.252433	15.43471	22.2866
261	14.06025	0.267439	6.721417	1.004436	7.419772	0.500973	29.97429	44.30336
265	14.30657	0.208031	6.609794	1.029528	6.801388	0.672904	29.62822	43.41987
267	15.07624	0.185861	6.530458	1.084105	6.796598	0.485883	30.15915	43.7075
271	14.97703	0.204619	6.109167	1.034744	6.200711	0.470342	28.99662	41.5537
275	15.50845	0.190329	6.269411	1.084948	6.287387	0.482659	29.82319	42.64121
279	20.02561	0.200124	5.95801	1.114163	5.787789	0.527203	33.6129	45.76392
283	21.64287	0.194063	6.960506	1.164982	6.24797	0.576167	36.78656	50.2417
289	21.3468	0.200939	6.11516	1.113538	5.954931	0.55395	35.28532	47.75905
293	22.2691	0	6.388289	1.192006	6.300044	0.595386	36.74483	49.81839
297	17.09265	0.13423	4.882799	0.913446	4.865237	0.465909	28.35427	38.47182
301	21.22959	0.411138	5.600651	1.052079	5.159792	0.588128	34.04138	45.37342

Table N4E. Acid concentration in F1 (Train B)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	3.900384	0.34116	0.775198	0.087808	0.076633	0	5.181182	6.037267
3	7.107691	0.635293	2.147731	0.261914	0.430332	0	10.58296	13.07197
7	9.787217	1.225303	3.119015	0.446066	0.820748	0	15.39835	19.40936
11	11.4608	2.046913	2.842396	0.472894	1.01204	0.081947	17.91699	22.40644
13	12.66237	2.272556	2.278368	0.459044	1.228254	0.142575	19.04316	23.52147
17	14.09256	2.329745	2.206272	0.560334	1.553279	0.242499	20.98469	25.99716
21	14.75137	2.217529	2.043455	0.58795	2.108942	0.486918	22.19616	27.95912
25	15.63012	2.17567	2.224882	0.789508	2.773073	0.769219	24.36247	31.46894
29	14.69463	2.114261	2.276436	0.754403	2.74052	0.707061	23.28731	30.26246
31	14.67871	2.122354	2.420275	0.786357	2.68127	0.692633	23.3816	30.4102
34	14.94944	2.105537	2.634066	0.851174	2.963403	0.770149	24.27377	31.9003
38	14.82515	1.972595	3.141792	0.98922	3.445794	0.877875	25.25243	33.95266
42	13.05607	1.630747	3.964739	1.167391	3.970174	0.931743	24.72086	34.64549
46	17.2501	2.293968	4.552517	1.386161	4.108954	0.873139	30.46484	41.36495
50	11.58301	1.162574	5.104938	1.380332	6.249806	1.222067	26.70273	40.21635
54	11.10142	0.976206	5.125176	1.394535	7.180808	1.295821	27.07397	41.62104
58	13.02786	0.964243	5.31878	1.398699	7.796142	1.38411	29.88983	45.33822
62	13.05979	0.968833	5.063397	1.334542	7.245077	1.284396	28.95603	43.4584
67	15.54957	1.047739	4.169566	1.121039	6.066878	1.170673	29.12547	41.43975
70	14.1577	0.997599	4.587651	1.222953	6.743991	1.237443	28.94733	42.43234
75	16.26692	1.035173	3.810274	1.019668	5.534286	1.090356	28.75668	40.05389
79	17.0216	1.0535	3.774525	1.006328	5.331937	1.053308	29.2412	40.24842
83	17.56119	1.035575	3.582302	0.940253	4.954973	0.988137	29.06243	39.38509
87	18.24769	1.045382	3.502357	0.912363	4.713447	0.94141	29.36265	39.29369
91	17.84141	0.975167	3.253101	0.830671	4.32087	0.872467	28.09369	37.24165
96	18.27475	0.973987	3.204099	0.826363	4.132075	0.837934	28.24921	37.11514
100	18.50516	0.94089	3.078488	0.774695	3.902248	0.795788	27.99726	36.41721
104	19.37709	0.969596	3.238311	0.698981	3.895193	0.786059	28.96523	37.42247
108	19.27361	0.924671	3.057841	0.774646	3.695784	0.751341	28.4779	36.60292
112	19.22269	0.933569	3.156141	0.573162	3.719374	0.762441	28.36738	36.41997
116	17.51237	0.820998	2.726421	0.482155	3.275751	0.687566	25.50526	32.55826
121	18.96452	0.874573	3.506797	0.523668	3.382112	0.695859	27.94752	35.73685
125	17.71418	0.797835	2.63576	0.458068	3.017348	0.637416	25.26061	31.88148
129	18.23857	0.817714	2.632291	0.485115	3.066626	0.633766	25.87408	32.5744
131	14.62822	0.543498	1.80559	0.260706	1.951815	0.372733	19.56256	23.85076
133	17.40447	0.767484	2.424111	0.436667	2.817024	0.585155	24.43491	30.59692
137	17.59717	0.759054	2.567459	0.448835	2.845069	0.584208	24.80179	31.10163
141	17.09227	0.711918	2.388049	0.322495	2.579675	0.549889	23.6443	29.35672
145	17.70374	0.739581	2.56232	0.5809	2.647911	0.555381	24.78983	30.95306
149	15.9537	0.669918	2.071224	0.382823	2.120697	0.47586	21.67422	26.61967
153	16.53061	0.723945	2.233577	0.411796	2.297101	0.54501	22.74204	28.12319
157	16.45562	0.725168	2.278052	0.396123	2.133805	0.456777	22.44555	27.56403
161	16.31149	0.746355	2.320136	0.390613	2.106997	0.446698	22.32229	27.43349
165	12.26209	0.523767	1.864194	0.30832	1.736587	0.360241	17.0552	21.17264

169	12.51302	0.549438	1.93916	0.322843	1.839094	0.364926	17.52848	21.83845
177	11.28157	0.459707	2.014929	0.315474	1.734825	0.322679	16.12918	20.2851
181	13.70621	0.712963	2.025429	0.321089	1.716421	0.381788	18.8639	23.19165
185	11.76919	0.529679	2.120535	0.322175	1.735532	0.328661	16.80577	21.08007
189	11.36546	0.49775	2.099626	0.322385	1.733804	0.32352	16.34254	20.58053
193	10.94567	0.467012	2.054586	0.392043	1.664341	0.305768	15.82942	19.9904
197	11.29986	0.511075	2.045934	0.404924	1.715659	0.328646	16.3061	20.57381
201	10.38229	0.441277	1.873704	0.366222	1.575932	0.290469	14.9299	18.81538
205	11.23537	0.502967	2.047584	0.416589	1.707659	0.32512	16.23529	20.49858
209	10.74125	0.448073	1.914037	0.381343	1.580168	0.292612	15.35749	19.29551
213	9.831177	0.377038	1.610996	0.240586	1.320459	0.245189	13.62544	16.85567
217	9.405313	0.332872	1.620398	0.333073	1.358906	0.242141	13.2927	16.63266
221	8.964777	0.303898	1.577892	0.242507	1.359276	0.234867	12.68322	15.88961
225	9.023047	0.254174	1.517787	0.239142	1.32017	0.223475	12.57779	15.66237
229	7.953057	0.214219	1.225671	0.182989	1.03136	0.195622	10.80292	13.26982
233	7.409819	0.199581	1.124406	0.177279	0.971806	0.176272	10.05916	12.35589
237	7.121462	0.176634	1.062722	0.184523	1.05422	0.152359	9.75192	12.06152
241	7.164983	0.19172	1.079413	0.183504	1.069873	0.16178	9.851271	12.20611
245	8.606832	0.236843	1.634079	0.346587	1.464035	0.202881	12.49126	15.87788
249	6.142564	0.169615	0.830454	0.145047	0.871047	0.129706	8.288432	10.17107
253	6.77493	0.184594	1.062939	0.159747	0.942778	0.153714	9.278702	11.45118
257	5.318493	0.133381	0.805094	0.133817	0.789036	0.124595	7.304416	9.049069
261	6.192734	0.224434	0.883216	0.158884	0.908309	0.232798	8.600375	10.71897
265	5.616354	0.12021	0.80203	0.150234	0.892613	0.145671	7.727113	9.615379
267	4.610335	0	0.590087	0.135473	0.80765	0.132823	6.276367	7.843876
271	4.163426	0	0.568247	0.139656	0.815162	0.13238	5.81887	7.381924
275	4.180052	0.149012	0.754266	0.152271	0.846067	0.156296	6.237965	8.068126
279	4.107389	0.182274	0.839249	0.148343	0.522544	0.177599	5.977397	7.556924
283	6.262954	0.214711	0.813817	0.142036	0.382093	0	7.815611	9.02439
289	5.385317	0.268874	0.709746	0.143102	0.130945	0	6.637983	7.527385
293	5.028779	0.196861	0.567956	0.108316	0.484241	0	6.386153	7.491405
297	4.248298	0.262303	0.449288	0	0	0	4.959888	5.390805
301	5.131561	0.31999	0.793804	0.238433	0.360355	0	6.844143	8.146849

Table N4F. Acid concentration in F2 (Train B)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	5.659358	0.476177	1.190436	0.097818	0.156652	0	7.580441	8.882675
3	6.23974	0.54263	1.377094	0.105745	0.172327	0	8.437537	9.92397
7	5.715857	0.507272	1.281531	0.094937	0.214126	0	7.813723	9.252283
11	5.126854	0.466654	0	0.085103	0.238641	0	5.917252	6.450807
13	5.292671	0.488714	1.220672	0.095592	0.2718	0	7.36945	8.819246
17	9.912809	1.562484	2.789345	0.776033	1.672065	0.266729	16.97946	22.43088
21	11.71433	2.219434	4.057933	1.191874	2.933148	0.527685	22.64441	31.31054
25	13.19156	2.386395	4.562354	1.330116	3.365984	0.621176	25.45758	35.25253
29	14.19341	2.459968	4.67789	1.36161	3.676962	0.708233	27.07808	37.45202
31	14.0232	2.369516	4.551089	1.324696	3.656596	0.703922	26.62902	36.81464
34	14.81383	2.367498	4.961169	1.404129	3.881335	0.783695	28.21165	39.09502
38	14.25388	2.16881	4.169176	1.311559	3.834427	0.82175	26.5596	36.71057
42	15.53307	2.150333	4.172898	1.281493	3.75269	0.783521	27.674	37.66029
46	18.12906	2.324899	4.56972	1.380051	4.047746	0.849594	31.30107	42.12783
50	20.06858	2.442328	4.876664	1.457966	4.268134	0.906327	34.02	45.48928
54	19.50462	2.269817	4.723108	1.386243	4.199899	0.899579	32.98326	44.12457
58	22.43315	2.30036	4.985098	1.410975	4.270954	0.888049	36.28858	47.71501
62	23.15291	2.324893	4.986981	1.403322	4.257701	0.893797	37.0196	48.44395
67	24.87782	2.343787	5.142255	1.426329	4.288741	0.854525	38.93345	50.48415
70	25.43281	2.342744	5.021309	1.388052	4.115262	0.822617	39.12279	50.32932
75	26.15216	2.218716	5.016183	1.365412	4.125055	0.824912	39.70244	50.84639
79	26.74166	2.25425	5.07717	1.371908	4.142606	0.823676	40.41127	51.63694
83	25.37292	2.115024	4.820066	1.298278	3.935661	0.785776	38.32772	48.97818
87	26.55871	2.118246	4.910474	1.309744	4.057966	0.810641	39.76578	50.65352
91	26.05355	2.064772	4.908227	1.282001	3.978074	0.787327	39.07395	49.7985
96	26.23172	1.968695	4.754801	1.254473	3.908418	0.763793	38.8819	49.3301
100	28.29557	1.906519	4.967248	1.334984	4.14666	0.7972	41.44818	52.39047
104	33.37531	2.005644	5.539067	1.38929	4.611637	0.859077	47.78003	59.79321
108	32.20541	1.903727	5.299615	1.350453	4.517951	0.832291	46.10945	57.74203
112	31.17761	1.833397	5.109583	0.935627	4.380035	0.833003	44.26925	55.21158
116	29.08116	1.644144	4.718762	0.863933	4.080303	0.773898	41.1622	51.29038
121	28.24133	1.525713	4.465188	0.806316	3.929984	0.75185	39.72038	49.3792
125	31.25394	1.623284	4.922676	0.891413	4.278492	0.806823	43.77663	54.31843
129	30.93335	1.5721	4.827031	0.953767	4.160718	0.78445	43.23142	53.58806
131	30.85198	1.519334	4.753221	0.86075	4.159571	0.795275	42.94013	53.14917
133	29.16445	1.513187	4.472799	0.816363	3.946376	0.753621	40.66679	50.35533
137	28.14849	1.397322	4.335056	0.805803	3.919195	0.742227	39.34809	48.83751
141	28.07535	1.315498	4.256449	0.770825	3.833309	0.755621	39.00705	48.29863
145	26.85033	1.20903	4.054525	0.977141	3.584529	0.724873	37.40043	46.38907
149	25.34068	1.133081	3.885296	0.929021	3.401893	0.70269	35.39267	43.96269
153	16.20447	0.642967	3.113535	0.628815	2.426466	0.465112	23.48136	29.69076
157	17.29628	0.723542	3.168842	0.653158	2.512881	0.489881	24.84458	31.27212
161	22.22101	0.963537	3.769137	0.837498	3.131043	0.648595	31.57082	39.54683
165	16.52164	0.699693	3.074472	0.636054	2.452088	0.48247	23.86641	30.1271

169	18.43452	0.805961	3.282675	0.709818	2.702388	0.565102	26.50046	33.38841
177	18.1465	0.801016	3.331926	0.686055	2.589134	0.526264	26.0809	32.81196
181	15.5946	0.66475	3.024489	0.599768	2.324995	0.43438	22.64298	28.62629
185	18.26021	0.820089	3.369159	0.680861	2.537745	0.507224	26.17528	32.85823
189	18.07982	0.829861	3.324244	0.670444	2.497604	0.491792	25.89376	32.47804
193	18.01811	0.821192	3.282985	0.658466	2.464377	0.480007	25.72514	32.216
197	16.80163	0.772179	3.108277	0.638575	2.363306	0.454554	24.13852	30.32766
201	17.16323	0.789137	3.071986	0.618721	2.326682	0.448477	24.41823	30.52458
205	16.13275	0.688897	2.891974	0.595067	2.263529	0.420382	22.9926	28.80612
209	17.40831	0.782061	3.069095	0.630862	2.391527	0.450663	24.73251	30.91718
213	16.56465	0.718622	2.837093	0.586543	2.192592	0.403963	23.30346	28.98775
217	15.84987	0.630555	2.704689	0.572596	2.120871	0.383664	22.26224	27.70169
221	15.31422	0.603005	2.596695	0.551308	2.053812	0.368083	21.48713	26.72847
225	15.61831	0.533908	2.530666	0.547505	2.024892	0.359726	21.61501	26.73654
229	14.46221	0.501447	2.334686	0.508093	1.883575	0.335871	20.02589	24.77868
233	13.30657	0.468877	2.143006	0.471505	1.732341	0.313011	18.43531	22.81549
237	12.22028	0.438033	1.995612	0.443278	1.669326	0.281892	17.04843	21.18028
241	12.17244	0.432507	2.015367	0.455715	1.716114	0.281549	17.07369	21.27817
245	11.03409	0.436999	1.904006	0.434149	1.611582	0.270897	15.69173	19.67419
249	7.238989	0.301547	1.272103	0.327046	1.089361	0.170134	10.39918	13.09819
253	11.17769	0.479128	2.106521	0.454932	1.649956	0.284549	16.15278	20.3724
257	4.959349	0.191579	0.831036	0.153479	0.81332	0.125445	7.074206	8.906296
261	9.771737	0.427438	1.733698	0.421912	1.554631	0.251767	14.16118	17.92336
265	9.238088	0.401636	1.78786	0.443208	1.622968	0.280938	13.7747	17.69127
267	8.164189	0.344077	1.711839	0.439026	1.610707	0.266226	12.53606	16.33938
271	4.353018	0.249109	1.141634	0.291513	1.083758	0.175985	7.295017	9.846023
275	5.258982	0.393617	1.589564	0.43038	1.583654	0.276573	9.532772	13.24365
279	4.994172	0.442475	1.467408	0.468436	1.22208	0.244324	8.838895	12.09296
283	4.884853	0.493857	1.472138	0.403699	1.202195	0.266355	8.723098	11.94788
289	6.758325	0.626606	1.072994	0.4402	0.306281	0.170905	9.375312	11.33575
293	7.00519	0.668571	0.84171	0.391911	0.166136	0	9.073519	10.49028
297	6.263697	0.808704	0.452655	0.232745	0.550436	0	8.308237	9.774679
301	10.08688	0.8429	1.470166	0.407876	0.950702	0.167071	13.9256	16.91594

Table N4G. Acid concentration in F3 (Train B)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	3.739602	0.31255	0.920447	0	0.148904	0	5.121504	6.065157
3	8.242604	0.682963	2.379649	0.268362	0.455415	0	12.02899	14.73545
7	9.584164	1.014799	3.180682	0.397851	0.751989	0	14.92948	18.77777
11	14.022	2.12961	3.98043	0.589691	1.443338	0	22.16507	27.96542
13	12.58493	2.087538	3.130842	0.583051	1.570698	0.148204	20.10526	25.59424
17	12.95788	2.164935	3.114506	0.572411	1.576516	0.16034	20.54658	26.06785
21	12.88891	2.085815	2.841791	0.57991	1.765434	0.254944	20.41681	26.03434
25	14.70631	2.097765	2.659584	0.679948	2.53476	0.571772	23.25014	30.03899
29	13.83381	1.952479	2.768595	0.6964	2.727599	0.696782	22.67566	29.85049
31	15.94785	2.185011	3.162494	0.895598	3.169005	0.824019	26.18397	34.54281
34	14.93574	1.706448	2.489101	0.711209	3.131565	0.903708	23.87777	31.44599
38	17.23609	2.017754	2.929498	0.895497	3.395827	0.95507	27.42973	35.95415
42	13.53053	1.600417	2.367541	0.646153	2.772235	0.783991	21.70087	28.55045
46	16.85748	1.873229	2.734997	0.830671	3.161809	0.895566	26.35375	34.299
50	17.57231	1.853421	2.758386	0.840529	3.143639	0.875027	27.04331	34.96237
54	20.94278	2.050388	2.992459	0.907727	3.413781	0.967973	31.2751	39.90657
58	22.21333	2.136079	3.19598	0.950997	3.625311	1.034116	33.15581	42.313
62	20.83744	1.940726	2.99177	0.896773	3.338258	0.942082	30.94705	39.41009
67	22.81272	2.010163	3.150776	0.942306	3.401971	0.961721	33.27966	42.01902
70	21.56144	1.805792	2.918236	0.867205	3.109855	0.870111	31.13264	39.13019
75	26.38016	2.071945	3.496756	1.003922	3.506802	0.966982	37.42657	46.61391
79	27.34269	2.101044	3.657304	1.033555	3.517799	0.936906	38.5893	47.90593
83	28.97859	2.133339	3.817294	1.046975	3.510894	0.931613	40.4187	49.86176
87	30.20379	2.133189	3.894207	1.067724	3.601538	0.928541	41.82899	51.43964
91	31.64367	2.175457	4.064408	1.105883	3.563111	0.89457	43.44709	53.15
96	32.04147	2.159883	4.018693	1.073552	3.447124	0.865415	43.60614	53.08203
100	33.56654	2.141323	4.095004	1.077279	3.450362	0.862132	45.19264	54.71883
104	34.62772	2.125445	4.264458	1.111036	3.553706	0.862152	46.54452	56.32606
108	36.00079	2.175867	4.359946	1.128355	3.532947	0.826498	48.02441	57.84669
112	30.80497	1.879352	3.792442	0.717143	3.085896	0.735939	41.01575	49.34613
116	32.5517	1.960213	4.275558	0.787911	3.453803	0.788694	43.81788	53.0463
121	33.52138	1.991681	4.542546	0.827477	3.612088	0.817647	45.31282	54.98394
125	34.50235	2.043182	4.842377	0.861794	3.744265	0.797041	46.79101	56.8445
129	35.4033	2.052536	5.005679	0.882837	3.854416	0.80996	48.00873	58.33478
131	34.18446	1.989726	5.002345	0.886127	3.791291	0.79933	46.65328	56.87229
133	33.49001	1.910709	5.050821	0.906291	3.821048	0.781644	45.96052	56.20914
137	33.06972	1.876082	5.007854	0.867287	3.759773	0.753952	45.33466	55.40375
141	33.66948	1.899927	4.851964	0.862357	3.683462	0.778387	45.74558	55.65811
145	31.68392	1.636259	4.448527	1.101191	3.371882	0.685356	42.92713	52.21587
149	31.14843	1.7448	4.347689	1.073958	3.271903	0.720615	42.30739	51.48027
153	31.22419	1.722568	4.338641	1.072853	3.317212	0.733974	42.40944	51.63011
157	23.27663	1.250161	4.145227	0.897589	2.838938	0.557111	32.96565	40.96134
161	30.18436	1.651968	4.311086	1.061437	3.320787	0.739213	41.26885	50.43921
165	26.53171	1.437774	3.920304	0.953763	3.048575	0.680548	36.57268	44.91427

169	24.81896	1.364214	3.772516	0.914556	2.948	0.668049	34.48629	42.5328
177	21.27625	1.035673	4.360375	0.893878	2.881352	0.523876	30.9714	39.02954
181	23.99774	1.332837	3.952397	0.900148	2.843623	0.635223	33.66197	41.65749
185	22.53654	1.230176	3.918458	0.870899	2.814982	0.618185	31.98924	39.83873
189	22.15281	1.091652	4.200669	0.891759	2.862014	0.558342	31.75725	39.7454
193	21.71086	1.066848	4.156152	0.868649	2.828516	0.555773	31.18679	39.07269
197	21.13807	1.023649	4.151847	0.867953	2.828578	0.544237	30.55433	38.40422
201	21.82801	0.99987	4.190071	0.866095	2.833442	0.532461	31.24995	39.10686
205	20.93435	1.074342	4.108061	0.866454	2.771346	0.562757	30.31731	38.11688
209	20.66633	1.057861	4.114851	0.865566	2.772569	0.556958	30.03414	37.82493
213	21.37248	0.955853	4.086182	0.851924	2.746332	0.512315	30.52509	38.15951
217	20.81452	0.853227	3.941474	0.822548	2.699383	0.485263	29.61641	36.9957
221	21.45461	0.882471	4.03487	0.840705	2.74363	0.49528	30.45157	37.98553
225	21.20325	0.784773	3.892893	0.813447	2.65179	0.46794	29.81409	37.05069
229	19.4086	0.690805	3.49829	0.736391	2.400417	0.426	27.1605	33.68795
233	19.69749	0.700956	3.496304	0.736294	2.397601	0.429898	27.45854	33.99064
237	10.18247	0.354159	1.848049	0.361759	1.296434	0.21163	14.2545	17.68764
241	18.54727	0.617248	3.274717	0.701829	2.309588	0.385458	25.83611	31.99688
245	17.44051	0.599341	3.192885	0.669126	2.218114	0.359587	24.47956	30.4154
249	15.06717	0.529745	2.729375	0.58946	1.915079	0.31642	21.14725	26.27589
253	19.09935	0.669724	3.371578	0.746992	2.365831	0.393879	26.64735	33.01053
257	7.594055	0.234715	1.305365	0.231892	1.011112	0.108459	10.4856	12.93598
261	17.66196	0.556755	3.049038	0.652103	2.173864	0.357542	24.45126	30.20248
265	17.8512	0.499653	3.062708	0.663258	2.161386	0.349298	24.5875	30.31153
267	17.87342	0.50678	3.149371	0.697328	2.263158	0.373981	24.86403	30.82181
271	16.45781	0.441451	2.934246	0.665777	2.119326	0.338783	22.9574	28.50983
275	17.14225	0.489074	3.091625	0.70044	2.330851	0.365915	24.12016	30.09481
279	21.54698	0.479758	2.852549	0.667634	2.005617	0.336625	27.88916	33.28162
283	20.78326	0.454079	2.432085	0.649352	2.016263	0.35891	26.69395	31.79947
289	22.49628	0.492103	2.403046	0.708431	2.198362	0.390214	28.68844	34.07717
293	4.504188	0.33565	0.979218	0.20032	1.57852	0.135115	7.733011	10.60348
297	22.23502	0.468575	2.38366	0.727006	2.307545	0.381142	28.50295	33.99092
301	20.33489	0.624941	2.636546	0.689065	2.11764	0.390521	26.79361	32.29743

Table N4H. Acid concentration in F4 (Train B)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	5.055365	0.447587	1.117437	0.086435	0.140771	0	6.847594	8.058379
3	9.1446	1.015411	2.140815	0.131653	0.171449	0	12.60393	14.85328
7	11.66917	1.819278	2.729479	0.326963	0.496289	0	17.04118	20.57211
11	12.69589	1.97336	2.518925	0.480544	1.210328	0.206058	19.0851	23.68382
13	13.45949	1.998993	2.529451	0.580765	1.714201	0.337371	20.62027	26.02566
17	14.70904	2.137265	2.742308	0.674324	2.125089	0.436153	22.82418	29.08232
21	13.08067	1.814991	2.3529	0.620754	2.152987	0.485297	20.5076	26.39371
25	15.14692	2.063982	2.711499	0.760721	2.700167	0.628844	24.01213	31.14284
29	14.85616	1.811879	2.489367	0.731109	3.063564	0.778121	23.7302	31.13823
31	14.32502	1.759738	2.426282	0.717742	2.998848	0.764193	22.99182	30.23484
34	16.20012	2.323774	2.644574	0.772561	3.120971	0.786889	25.84888	33.69245
38	16.48188	1.990789	2.836981	0.81986	3.31195	0.86052	26.30198	34.47734
42	13.89259	1.518497	2.276483	0.646289	2.66118	0.683116	21.67815	28.1901
46	21.53365	2.192093	3.441794	0.922519	3.680248	0.955073	32.72538	42.01749
50	22.13843	2.161675	3.498903	0.923489	3.56699	0.915825	33.20532	42.35823
54	22.04098	2.053935	3.413449	0.881806	3.376282	0.858809	32.62527	41.36216
58	23.02711	2.126858	3.594813	0.916601	3.411344	0.860581	33.93731	42.90513
62	24.71805	2.13053	3.631295	0.913697	3.291078	0.830972	35.51562	44.34538
67	25.29945	2.148208	3.693377	0.925496	3.299824	0.826134	36.19249	45.08835
70	23.84288	2.020724	3.595138	0.915759	3.125676	0.777115	34.27729	42.79514
75	28.30789	2.186761	3.926848	0.956732	3.272678	0.79366	39.44456	48.48463
79	28.86915	2.156944	3.969763	0.954267	3.20719	0.775311	39.93262	48.89665
83	28.46563	2.079225	3.890973	0.926108	3.077379	0.743114	39.18243	47.85469
87	30.37234	2.140227	3.988346	0.957202	3.150591	0.764282	41.37299	50.27328
91	34.7054	2.348657	4.478043	1.039677	3.44114	0.804135	46.81705	56.58823
96	30.2727	1.997451	3.903509	0.927609	2.995549	0.720534	40.81735	49.35241
100	32.12898	2.061767	4.003511	0.941399	2.998523	0.714306	42.84848	51.49105
104	32.69517	2.024729	4.010187	0.943218	3.01702	0.700357	43.39068	52.02768
108	33.07748	2.020973	4.030699	0.953346	3.037453	0.718494	43.83844	52.54145
112	31.16524	2.471977	4.568654	0.866735	4.193276	0.992957	44.25884	55.01566
116	35.22846	1.997123	4.283951	0.78189	3.402935	0.708494	46.40285	55.4992
121	34.05097	1.816579	4.224038	0.769384	3.265246	0.715166	44.84138	53.66773
125	32.65632	1.697579	4.012407	0.713016	2.955124	0.66927	42.70372	50.89117
129	32.72162	1.751197	4.038923	0.730554	3.149412	0.759223	43.15093	51.71113
131	32.17583	1.74506	4.031602	0.709472	2.929541	0.693437	42.28494	50.50371
133	34.82207	1.714426	4.213664	0.731961	2.984407	0.613279	45.07981	53.40002
137	33.11589	1.77524	4.300739	0.747213	3.099483	0.658031	43.69659	52.29298
141	38.90226	2.178791	4.933686	0.890878	4.065612	0.83006	51.80129	62.38994
145	32.41942	1.465236	3.835573	0.909913	2.732075	0.646196	42.00842	49.88669
149	31.53281	1.639955	3.826027	0.903834	2.699124	0.608267	41.21002	49.06809
153	32.44402	1.670973	3.967033	0.934411	2.778004	0.613265	42.4077	50.49746
157	31.70208	1.62376	3.953161	0.93113	2.773261	0.613435	41.59683	49.64847
161	28.58589	1.452979	3.79729	0.877653	2.707004	0.604279	38.02509	45.76163
165	28.98181	1.459183	3.734152	0.868264	2.676666	0.60218	38.32226	45.9736

169	26.21854	1.318559	3.607125	0.838331	2.585002	0.582786	35.15034	42.50198
177	21.98203	0.968771	4.125411	0.816235	2.624498	0.491447	31.00839	38.48848
181	23.20934	1.184166	3.210259	0.715036	2.24341	0.535204	31.09742	37.57475
185	22.90198	1.13163	3.839283	0.817908	2.615295	0.562137	31.86823	39.2916
189	24.47157	1.097619	4.497127	0.90295	2.874503	0.538619	34.38239	42.58432
193	24.62234	1.122833	4.342076	0.89031	2.810355	0.552664	34.34058	42.3803
197	21.58037	1.082675	3.653822	0.770639	2.413797	0.53417	30.03547	37.01393
201	24.00527	1.083415	4.285545	0.878046	2.766173	0.542926	33.56137	41.47398
205	23.91079	1.060867	4.25993	0.876959	2.752791	0.537205	33.39855	41.26136
209	23.72856	1.078696	4.257654	0.885169	2.759605	0.53779	33.24747	41.13151
213	24.02837	1.026021	4.283716	0.875759	2.774666	0.51162	33.50015	41.357
217	24.72096	1.030608	4.516977	0.915411	2.922404	0.543427	34.64979	42.90619
221	23.6277	0.973991	4.283099	0.870955	2.768745	0.507175	33.03167	40.84985
225	23.54368	0.879517	4.189719	0.856754	2.701902	0.49438	32.66595	40.27864
229	24.55028	0.89696	4.240221	0.86937	2.730751	0.488745	33.77633	41.46851
233	23.16183	0.853312	4.008393	0.824406	2.620938	0.469634	31.93851	39.26607
237	20.71468	0.710948	3.64948	0.751532	2.439556	0.409167	28.67536	35.35473
241	22.16948	0.773404	3.890851	0.800786	2.597475	0.43023	30.66223	37.77866
245	16.14459	0.572577	2.902795	0.652191	1.959789	0.322045	22.55399	27.93583
249	22.13024	0.815757	3.762297	0.774852	2.537396	0.417407	30.43795	37.37807
253	17.79701	0.60805	3.274697	0.664754	2.194292	0.353867	24.89267	30.8539
257	18.87858	0.625821	3.425024	0.71325	2.313568	0.367744	26.32399	32.58828
261	21.05696	0.852759	3.812891	0.810162	2.594518	0.405242	29.53253	36.6019
265	19.74638	0.597505	3.703374	0.791305	2.583713	0.41378	27.83606	34.70119
267	17.84197	0.454885	3.337969	0.72501	2.361328	0.454989	25.17615	31.47236
271	15.69057	0.423122	2.957269	0.684758	2.090431	0.33306	22.17921	27.71912
275	18.57319	0.494246	3.505258	0.773164	2.509884	0.400074	26.25582	32.82475
279	24.43385	0.533239	3.466844	0.762671	2.447422	0.450002	32.09403	38.64026
283	21.81057	0.526352	3.585831	0.807214	1.958431	0.530334	29.21873	35.45694
289	21.53277	0.333084	3.486492	0.823206	1.975654	0.428891	28.5801	34.57866
293	21.81871	0.465754	3.593923	0.834579	2.001893	0.432717	29.14758	35.32101
297	22.30628	0.448984	3.57849	0.857535	2.124174	0.444369	29.75983	36.08154
301	22.67738	0.682998	3.579907	0.851643	2.04474	0.448144	30.28481	36.62021

Table N4I. Acid concentration in F1 (Train C)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	3.98708	0.274235	0.707795	0	0.191063	0	5.160174	5.982951
4	7.803904	0.539119	1.621909	0	0.249325	0	10.21426	11.85921
8	10.08067	0.796729	2.900066	0.296093	0.594979	0	14.66854	17.96647
12	9.608364	1.000004	3.424371	0.430653	1.04205	0	15.50544	19.86009
16	8.846156	1.16102	3.575707	0.537216	1.740214	0.098143	15.95846	21.449
20	8.327929	1.371527	3.741966	0.65407	2.557414	0.211024	16.86393	23.67634
24	8.359066	1.529347	3.790469	0.718069	3.07795	0.294563	17.76946	25.39798
28	7.913105	1.650257	3.736563	0.801111	3.745745	0.417215	18.264	26.84219
32	9.246531	1.97349	4.373585	0.940846	4.115544	0.442816	21.09281	30.81177
36	9.84531	2.139751	4.676606	1.091983	4.988298	0.557659	23.29961	34.51246
40	9.216288	2.027739	4.335105	1.051819	4.678059	0.55227	21.86128	32.41017
44	9.070958	2.023819	4.34125	1.092045	5.166224	0.626002	22.3203	33.51922
48	10.58246	2.124148	4.357939	1.132399	5.365162	0.880535	24.44264	36.25056
52	9.617724	1.865907	3.899622	1.067117	5.080655	0.838292	22.36932	33.3297
56	10.88377	2.022566	4.380138	1.258184	6.155295	1.080491	25.78045	38.76126
60	9.26808	1.740641	3.745025	1.100084	5.369469	0.784508	22.00781	33.08125
64	10.0021	1.823067	4.018396	1.158788	5.301594	0.774471	23.07842	34.34905
68	9.49086	1.718377	4.063923	1.136762	5.232738	0.758537	22.4012	33.54669
72	7.997974	1.443157	3.222373	0.994802	4.64702	0.673229	18.97855	28.55443
76	8.517499	1.52269	3.511585	1.058642	5.010821	0.72895	20.35019	30.67845
80	9.576499	1.662704	3.76634	1.156106	5.104935	0.729329	21.99591	32.75396
84	7.789492	1.441152	3.057601	0.971696	4.489274	0.649757	18.39897	27.63948
88	8.511691	1.489244	3.761193	1.087863	4.629363	0.73124	20.2106	30.32201
92	8.060566	1.457359	3.199716	1.046044	4.482156	0.684689	18.93053	28.3804
96	7.831845	1.60348	4.072778	1.33211	5.577861	0.592809	21.01088	32.46302
100	8.091035	1.378521	3.217256	1.095088	4.501179	1.108146	19.39123	29.39121
104	7.597003	1.320056	2.993898	1.034599	4.063788	0.719994	17.72934	26.5596
108	7.843457	1.686631	4.028807	1.342233	4.37882	0.44363	19.72358	29.72692
112	6.084784	1.165429	2.281323	0.954605	3.581936	0.602965	14.67104	22.20331
116	5.579222	1.106806	2.340024	0.923059	3.34891	0.565924	13.86395	21.09006
120	5.687091	1.080736	2.250982	0.879244	2.911693	0.523399	13.33315	19.92661
124	6.120954	1.056312	2.140177	0.834666	2.454118	0.476181	13.08241	19.00073
128	7.507302	1.189607	2.443111	0.911261	2.431975	0.436923	14.92018	21.10889
132	6.979063	1.098025	2.241734	0.856478	2.272502	0.417112	13.86491	19.6281
136	6.448581	1.007247	2.038988	0.81603	2.116633	0.396937	12.82442	18.17797
140	7.513592	1.062003	2.115403	0.80146	1.917009	0.723388	14.13286	19.72703
144	7.329801	1.028705	2.081285	0.788595	1.979462	0.510934	13.71878	19.07615
148	6.717797	1.003489	1.968697	0.752063	1.741344	0.415132	12.59852	17.46259
152	5.947542	0.989683	1.924851	0.746573	1.60634	0.375927	11.59091	16.2212
156	5.308959	1.018803	1.817549	0.720169	1.48252	0.339263	10.68726	15.05303
160	5.421227	1.079024	1.967161	0.74499	1.26579	0.347065	10.82526	15.12198
169	4.418963	1.019632	1.755016	0.676629	1.186039	0.640534	9.696813	14.02059
173	4.142378	0.96888	1.58389	0.593872	0.879648	0.373935	8.542603	12.00203

177	4.438739	0.98987	1.566109	0.615882	0.716123	0.299286	8.62601	11.83783
181	4.782727	0.970593	1.351317	0.537653	0.428603	0.21957	8.290462	10.86834
185	2.217417	0.448129	0.409225	0	0	0	3.074771	3.553777
189	4.287381	0.81986	0.854714	0.213214	0.187322	0	6.362491	7.70919
193	4.944813	0.940197	0.629546	0.34334	0.202983	0.291147	7.352025	9.074154
197	4.464019	0.890792	0.456532	0	0	0	5.811343	6.511581
201	4.48289	0.876993	0.403274	0	0	0	5.763158	6.420018
205	4.439454	0.885024	0.209392	0	0	0	5.533869	6.057192
209	4.152886	0.847352	0.170592	0	0	0	5.17083	5.650696
213	3.824036	0.78784	0	0	0	0	4.611876	4.946035
217	2.740322	0.812223	0	0.233513	0.14965	0.214151	4.14986	5.122595
221	2.146704	0.691181	0	0	0	0	2.837884	3.130039
225	2.016062	0.672464	0	0	0	0	2.688526	2.97271
229	1.709915	0.5951	0	0	0	0	2.305014	2.556442
233	1.624063	0.564284	0	0	0	0	2.188347	2.426757
237	1.349999	0.505085	0	0	0	0	1.855084	2.068397
241	1.332125	0.488147	0	0	0	0	1.820272	2.026453
245	1.170158	0.453885	0	0	0	0	1.624043	1.815696
249	1.471081	0.335997	0	0	0.286868	0.340338	2.434285	3.290219
253	1.397466	0.342216	0	0	0	0	1.739681	1.884611
257	1.346185	0.317648	0	0	0	0	1.663833	1.7984
261	1.208591	0	0	0	0	0	1.208591	1.209598
265	1.180116	0	0	0	0	0	1.180116	1.181099
269	1.09547	0	0	0	0	0	1.09547	1.096383
273	0.950645	0	0	0	0.283258	0.549319	1.783221	2.743255
277	0.870629	0	0	0	0	0	0.870629	0.871355
281	0.876542	0	0	0	0	0	0.876542	0.877273
285	0	0	0	0	0	0	0	0
289	0	0	0	0	0	0	0	0

Table N4J. Acid concentration in F2 (Train C)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	3.814876	0.27285	0.674769	0	0.202866	0	4.965361	5.776735
4	8.177211	0.469905	1.567798	0	0.242458	0	10.45737	12.028
8	9.68701	0.800055	2.314139	0.231943	0.408806	0	13.44195	16.06939
12	10.57864	1.069419	2.790655	0.30685	0.604499	0	15.35006	18.70574
16	9.088327	1.299765	2.875561	0.419339	1.175826	0.083889	14.94271	19.26849
20	8.947274	1.695154	3.096354	0.580458	1.927075	0.192689	16.439	22.16808
24	9.023297	1.956473	3.253137	0.648827	2.35127	0.265184	17.49819	24.05099
28	8.787975	2.164017	3.199311	0.772503	3.216378	0.419002	18.55919	26.38388
32	10.92434	2.848472	3.954903	0.966526	3.860222	0.481703	23.03617	32.62504
36	10.59453	2.899396	3.701257	0.995047	4.147528	0.580953	22.91871	32.80182
40	9.728464	2.699216	3.423816	0.936538	3.815958	0.552489	21.15648	30.31648
44	10.53541	2.874843	3.635273	1.05452	4.424655	0.653805	23.17851	33.44271
48	12.81278	2.984392	3.67271	1.123836	4.840327	0.776161	26.2102	37.20323
52	11.36401	2.407112	3.278699	1.109003	5.078175	0.888787	24.12579	34.97253
56	14.2527	2.793202	3.832318	1.380936	6.108018	1.062523	29.42969	42.39036
60	13.98675	2.621016	3.951653	1.408082	5.932168	1.003679	28.90335	41.64195
64	11.14303	2.087282	3.421097	1.251584	5.471444	0.970962	24.3454	35.80755
68	10.73387	2.216572	3.244214	1.177649	5.123627	0.914801	23.41073	34.29499
72	11.55917	2.06774	3.537589	1.271701	5.609106	1.00926	25.05457	36.8026
76	11.9347	2.100892	3.628358	1.329824	5.762212	1.018112	25.7741	37.82804
80	11.21797	1.947274	3.425756	1.295258	5.491003	0.958319	24.33558	35.78801
84	9.812991	2.15825	3.951443	1.490733	5.668899	0.67573	23.75805	35.70061
88	11.09882	1.939399	3.566778	1.293745	5.634767	1.029685	24.56319	36.34953
92	11.1201	1.9474	3.601888	1.317744	5.674226	1.060479	24.72184	36.63728
96	11.22487	1.882531	3.59933	1.303024	5.493857	0.93718	24.4408	35.97347
100	10.34281	1.825413	3.526043	1.310564	5.495617	1.016279	23.51673	35.07616
104	8.32043	2.016622	4.120871	1.569336	5.940645	0.73984	22.70774	35.14848
108	10.05645	1.9327	3.772399	1.48192	5.816291	1.03012	24.08988	36.38444
112	8.471912	1.709505	3.380564	1.320143	5.282379	0.995566	21.16007	32.32225
116	10.29964	1.956281	3.87917	1.596534	6.122923	1.056616	24.91116	37.75584
120	8.64008	1.640365	3.322457	1.677771	5.248255	0.964279	21.49321	32.83821
124	8.91276	1.619281	3.274933	1.336207	4.966164	0.943004	21.05235	31.71576
128	9.23417	1.588069	3.203438	1.279368	4.810617	0.896841	21.0125	31.33901
132	9.501831	1.594156	3.2109	1.287082	4.818788	0.89771	21.31047	31.66186
136	9.888425	1.624987	3.227308	1.259973	4.419023	0.76943	21.18915	30.95943
140	10.86727	1.719517	3.109845	1.359286	5.047756	0.878314	22.98199	33.60378
144	10.21507	1.611452	2.903888	1.254017	4.52347	0.817738	21.32564	31.02626
148	10.61838	1.623139	3.236149	1.264611	4.484463	0.821614	22.04835	31.96132
152	12.0385	1.735815	3.094306	1.304866	4.440124	0.786937	23.40055	33.20979
156	12.05808	1.67468	3.302841	1.282685	4.452149	0.805064	23.5755	33.52055
160	10.9255	1.504481	2.997452	1.148314	3.79608	0.691018	21.06284	29.75849
169	11.97387	1.52159	3.050176	1.180812	4.223268	0.749246	22.69896	31.99649
173	13.25741	1.609737	3.237904	1.25744	4.397363	0.803334	24.56319	34.35234

177	12.86922	1.575064	3.185133	1.254065	4.42413	0.786708	24.09432	33.83704
181	11.4304	1.55505	3.046622	1.253878	4.390709	0.812869	22.48953	32.12015
185	13.47079	1.933557	3.599173	1.545891	4.920214	0.80733	26.27695	37.2854
189	9.164888	1.515479	2.645671	1.149297	2.889215	0.512866	17.87742	25.14506
193	8.677908	1.540905	2.793601	1.163518	2.675239	0.505426	17.3566	24.51396
197	8.976389	1.668519	2.892596	1.185368	2.632696	0.513103	17.86867	25.13335
201	8.487249	1.732627	2.904307	1.147016	2.246343	0.464236	16.98178	23.7742
205	8.854545	1.76767	2.969905	1.142587	1.951534	0.450302	17.13654	23.65398
209	8.661295	1.862408	3.06794	1.124757	1.540672	0.46184	16.71891	22.90278
213	4.945675	1.685494	2.812646	1.0539	0.941289	0.259601	11.69861	16.67685
217	5.101168	1.761726	2.753952	1.078827	0.967862	0.259157	11.92269	16.94235
221	5.310042	1.792423	2.601653	0.996775	0.818086	0.235289	11.75427	16.41567
225	5.061349	1.68658	2.367349	0.914777	0.641951	0.201036	10.87304	15.01997
229	4.973709	1.623285	2.089499	0.855499	0.484088	0.168634	10.19471	13.85697
233	5.152907	1.675141	2.119613	0.872766	0.444978	0.165693	10.4311	14.10693
237	5.434268	1.741755	2.097735	0.899135	0.382444	0.14972	10.70506	14.33172
241	5.205065	1.619955	1.829738	0.810898	0.324337	0.133036	9.923029	13.14641
245	7.892358	1.51749	1.519167	0.604442	0.298845	0	11.8323	14.4209
249	8.12739	1.551341	1.502961	0.649363	0.274591	0	12.10565	14.71228
253	7.751886	1.453473	1.322273	0.592391	0.231527	0	11.35155	13.69105
257	7.308655	1.381255	1.234263	0.557588	0.196489	0	10.67825	12.85561
261	7.579062	1.408967	1.185961	0.543422	0	0	10.71741	12.64922
265	7.216971	1.322222	1.027538	0.484042	0	0	10.05077	11.77977
269	6.757519	1.282672	0.968205	0.44121	0	0	9.449606	11.08059
273	6.529971	1.207225	0.848546	0.434038	0	0	9.019781	10.52786
277	5.772169	1.096728	0.721279	0.383163	0	0	7.973338	9.298048
281	5.522233	1.053876	0.664027	0.34917	0	0	7.589306	8.82434
285	5.564262	1.028547	0.602831	0.322792	0	0	7.518433	8.675567
289	1.841021	0.32736	0	0	0	0	2.168381	2.30744

Table N4K. Acid concentration in F3 (Train C)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	1.830916	0.12617	0.299675	0	0.100057	0	2.356819	2.730039
4	4.005497	0.285835	0.700258	0	0.172257	0	5.163847	5.966056
8	7.160869	0.660715	2.081235	0.197948	0.5633	0	10.66407	13.2008
12	7.379416	0	2.280421	0.323999	0.68609	0	10.66993	13.31649
16	7.987583	0.957994	2.657595	0.420112	1.218412	0.07486	13.31656	17.37949
20	8.075384	1.103599	0	0.395199	1.506696	0.119552	11.20043	13.78771
24	8.278376	1.37159	2.892681	0.612369	2.346002	0.279515	15.78053	21.8107
28	8.516103	1.517739	3.013168	0.683566	2.948097	0.375901	17.05457	24.05618
32	8.154743	1.502131	2.939385	0.691835	3.01767	0.393067	16.69883	23.74403
36	8.383672	1.570601	3.162754	0.775356	3.536612	0.482927	17.91192	25.88299
40	10.65905	1.955612	3.975386	0.968868	4.402218	0.590599	22.55174	32.49225
44	8.108007	1.534493	3.18627	0.945019	4.127055	0.612404	18.51325	27.42727
48	9.94535	1.68718	3.451201	0.970592	4.479022	0.696174	21.22952	30.89649
52	9.693925	1.627165	3.420802	0.991104	4.689506	0.717966	21.14047	31.03068
56	9.267737	1.536778	3.309665	0.965311	4.553176	0.681469	20.31414	29.87445
60	9.453779	1.583165	3.394584	1.013895	4.775279	0.719982	20.94068	30.90876
64	9.814761	1.640276	3.570678	1.164637	5.015374	0.737263	21.94299	32.47506
68	9.982926	1.671206	3.630294	1.120696	5.109267	0.748759	22.26315	32.92456
72	9.609523	1.620146	3.516403	1.133721	4.971796	0.750203	21.60179	32.02747
76	10.23597	1.684823	3.694816	1.148742	5.129205	0.748839	22.64239	33.40235
80	10.82437	1.7958	3.840269	1.213007	5.22772	0.751383	23.65255	34.72951
84	10.17673	1.674457	3.750745	1.147457	5.040451	0.727525	22.51737	33.19075
88	10.5717	1.774085	3.857263	1.21223	4.983077	0.734354	23.13271	33.92936
92	11.2602	1.876552	4.127708	1.347589	6.201079	0.936916	25.75004	38.45086
96	11.94713	1.976378	4.280612	1.359806	5.597998	0.792198	25.95412	37.99802
100	11.14924	1.77685	3.796103	1.196449	5.116522	0.805709	23.84088	34.80966
104	11.94289	1.880211	3.999345	1.282596	5.442809	0.810203	25.35805	36.9478
108	9.739109	2.161115	4.85873	1.594749	5.820785	0.57838	24.75287	37.47846
112	13.09668	2.056906	4.382585	1.509074	6.320605	0.933089	28.29894	41.52788
116	12.87274	2.004989	4.337237	1.47029	6.004281	0.897106	27.58664	40.3445
120	12.97503	1.955359	4.208686	1.479758	6.195888	0.960072	27.77479	40.71012
124	12.805	1.858018	3.921576	1.383658	5.348603	0.821595	26.13845	37.66974
128	15.66796	2.112651	4.443866	1.59095	5.768021	0.805719	30.38917	43.01798
132	14.77434	1.964544	4.135243	1.506427	5.622999	0.755968	28.75952	40.8156
136	13.14911	1.808421	3.762218	1.393532	5.194243	0.864012	26.17154	37.46418
140	13.64661	1.813289	3.550182	1.337647	4.925115	0.72325	25.99609	36.63422
144	15.75187	1.982792	4.080602	1.431522	4.959651	0.697521	28.90396	40.08149
148	15.14579	1.857019	3.852118	1.390139	5.222503	0.741976	28.20954	39.46916
152	14.81473	1.813077	3.752636	1.349903	5.009978	0.731167	27.47149	38.36493
156	15.78707	1.875622	3.602612	1.405049	4.949157	0.689311	28.30882	39.0587
160	14.61218	1.718767	3.591859	1.289914	4.696671	0.700309	26.6097	36.92275
169	14.99246	1.793151	3.733585	1.323815	4.842544	0.715536	27.40109	38.0511
173	14.83014	1.70206	3.595593	1.289256	4.808505	0.732831	26.95838	37.42521

177	15.17738	1.717346	3.64625	1.311556	4.823511	0.716224	27.39227	37.91817
181	16.10887	1.781745	3.786634	1.354021	4.959203	0.741921	28.73239	39.59999
185	17.01611	1.312429	3.596695	1.327019	4.647612	0.672992	28.57285	38.66937
189	19.72942	2.008182	4.323874	1.57139	5.664663	0.741046	34.03857	46.33641
193	18.79459	1.855549	3.999886	1.405202	4.028545	0.518492	30.60226	40.43715
197	18.04003	1.759113	3.828142	1.357486	4.152837	0.547589	29.6852	39.48194
201	20.97207	1.98017	4.378385	1.574292	4.76034	0.576259	34.24151	45.40472
205	20.2094	1.831449	4.096721	1.454901	4.347867	0.589513	32.52985	42.89625
209	18.02476	1.778022	4.000897	1.432373	4.114881	0.605915	29.95685	39.98089
213	3.162836	1.6971	1.83113	1.491755	2.200967	0.439609	10.8234	17.07571
217	1.670123	1.492226	1.518394	1.31779	1.405226	0.240348	7.644107	12.3395
221	1.690748	1.559522	1.478701	1.2764	1.366089	0.236857	7.608318	12.2201
225	1.718587	1.72141	1.452887	1.267657	1.309554	0.219715	7.68981	12.26242
229	1.510897	1.626562	1.236133	1.024347	1.069623	0.184779	6.652341	10.51107
233	1.515661	1.669082	1.230583	0.983004	0.995329	0.178902	6.572562	10.32091
237	1.398551	1.606804	1.144994	0.900811	0.897952	0.160833	6.109944	9.570703
241	1.42215	1.621986	1.166064	0.911	0.872149	0.16335	6.156698	9.623414
245	1.32633	1.537422	1.117345	0.853565	0.792949	0.148531	5.776141	9.017905
249	1.964197	1.393889	0.993999	0.700028	0.774101	0	5.826215	8.583354
253	1.962023	1.320416	0.950006	0.657454	0.771628	0	5.661527	8.315206
257	1.790896	1.179091	0.896043	0.60388	0.643252	0	5.113162	7.482849
261	5.634329	1.385973	1.853385	0.873794	1.485741	0.321143	11.55436	16.22208
265	7.591135	1.564502	2.305373	0.974882	1.701752	0.332527	14.47017	19.87081
269	4.219575	1.057035	1.406931	0.613419	1.035456	0.23286	8.565275	11.95309
273	0.851775	0.262755	0	0	0	0	1.11453	1.225624
277	1.41581	0.349143	0	0	0	0	1.764953	1.912808
281	1.482973	0.352716	0	0	0	0	1.835688	1.985101
285	1.373899	0.294533	0	0	0	0	1.668432	1.793311
289	1.719626	0.360234	0	0	0	0	2.07986	2.232628

Table N4L. Acid concentration in F4 (Train C)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	3.063309	0.242782	0.512255	0	0.158573	0	3.976918	4.612881
4	7.784821	0.684691	1.265668	0	0.192956	0	9.928136	11.32238
8	7.378281	1.025487	3.251663	0.495606	1.610294	0	13.76133	18.67064
12	7.19837	1.139655	3.513006	0.557764	1.83156	0	14.24036	19.67566
16	6.297438	1.15921	3.650487	0.647948	2.332626	0.106236	14.19394	20.47948
20	6.168451	1.278175	3.742544	0.785801	2.774934	0.174562	14.92447	22.00594
24	5.055239	1.194408	3.159353	0.641875	2.842429	0.233115	13.12642	19.77079
28	5.105892	1.344324	3.246183	0.686244	3.225045	0.313529	13.92122	21.23613
32	5.529143	1.485065	3.665318	0.838374	3.385386	0.324695	15.22798	23.22222
36	6.353417	1.806396	4.283127	1.051919	4.423311	0.449917	18.36809	28.39002
40	5.822443	1.709323	3.895465	1.035218	4.740347	0.538994	17.74179	27.87939
44	6.017309	1.733698	3.908154	1.080524	4.739825	0.544973	18.02448	28.22941
48	7.509292	1.899303	4.113181	1.217766	5.538518	0.65967	20.93773	32.47568
52	6.188184	1.511145	3.473766	1.077296	4.898274	0.610231	17.7589	27.80843
56	6.454696	1.482519	3.486622	1.118918	5.015623	0.646518	18.2049	28.4587
60	8.532587	1.779643	4.004112	1.382994	6.056166	0.717974	22.47348	34.65979
64	8.647073	1.719875	4.09325	1.417818	5.994092	0.682075	22.55418	34.70088
68	7.335308	1.487775	3.553979	1.235838	5.361465	0.664298	19.63866	30.44127
72	7.019487	1.376203	3.312453	1.162169	5.017151	0.633021	18.52048	28.63216
76	7.585306	1.479916	3.500402	1.233908	5.360308	0.636181	19.79602	30.52113
80	7.822783	1.465337	3.510438	1.234416	5.319408	0.624279	19.97666	30.64539
84	7.562574	1.467857	3.450179	1.22612	5.143931	0.635422	19.48608	29.93096
88	7.605146	1.479317	3.437908	1.235873	5.146283	0.621485	19.52601	29.96186
92	7.538331	1.452301	3.374922	1.202706	5.016694	0.609956	19.19491	29.39208
96	8.305202	1.598907	3.578798	1.270862	5.239672	0.632409	20.62585	31.35698
100	9.05739	1.638156	3.686776	1.302739	5.189694	0.642709	21.51746	32.32984
104	9.662336	1.686697	3.817994	1.363781	5.54533	0.656924	22.73306	34.11248
108	6.987492	1.691366	4.137097	1.536785	5.593439	0.46376	20.40994	31.99322
112	9.231762	1.61024	3.538679	1.387418	5.488347	0.652259	21.90871	33.01346
116	9.529255	1.618825	3.722349	1.383522	5.457773	0.654911	22.36664	33.57178
120	10.53083	1.681655	3.843924	1.413644	5.481557	0.647962	23.59958	34.96246
124	10.58547	1.617555	3.702228	1.41166	5.464089	0.649481	23.43048	34.64775
128	11.85662	1.691847	3.820849	1.421088	5.329281	0.629647	24.74934	35.92321
132	13.28772	1.803903	4.087487	1.527219	5.563536	0.64934	26.91921	38.70085
136	11.99153	1.600109	3.628577	1.380754	5.144807	0.615655	24.36144	35.11008
140	12.25589	1.647565	3.742261	1.39764	5.006606	0.61366	24.66362	35.37775
144	13.36773	1.708835	3.810225	1.426332	5.047843	0.565332	25.9263	36.72772
148	13.32385	1.66327	3.76819	1.472367	5.651727	0.633911	26.51331	38.03639
152	15.99219	1.864039	4.136031	1.479943	4.998524	0.56949	29.04021	40.14015
156	13.40673	1.594099	3.617829	1.348693	4.833199	0.553219	25.35377	35.656
160	13.49818	1.604117	3.644576	1.339656	4.852344	0.603212	25.54209	35.93943
169	13.51657	1.587291	3.646442	1.346588	4.8571	0.58735	25.54134	35.92543
173	13.91727	1.585289	3.671392	1.357118	4.896725	0.593242	26.02104	36.48131

177	13.80116	1.547714	3.578024	1.317788	4.773583	0.570732	25.589	35.77283
181	14.68368	1.597849	3.71026	1.358672	4.839953	0.586099	26.77651	37.20225
185	20.06316	1.909368	4.628447	1.743374	5.926234	0.670485	34.94107	47.76557
189	16.4818	1.62463	3.886647	1.393445	3.957323	0.442028	27.78588	37.26358
193	16.50852	1.599976	3.890576	1.389333	4.122803	0.450069	27.96127	37.61444
197	17.72918	1.666432	4.097966	1.443466	4.191915	0.440954	29.56992	39.51099
201	16.26	1.541956	3.896268	1.40302	4.137804	0.454657	27.6937	37.36035
205	16.01447	1.492322	3.912335	1.423967	4.224276	0.452436	27.5198	37.2858
209	15.50831	1.486546	3.903806	1.428929	4.258319	0.45895	27.04486	36.85074
213	8.058617	1.286584	3.46113	1.376717	3.535591	0.311505	18.03014	26.43573
217	8.218767	1.352554	3.611418	1.410652	3.838091	0.335871	18.76735	27.69086
221	8.299255	1.417751	3.781276	1.600784	4.697652	0.435899	20.23262	30.51692
225	9.180273	1.604431	4.097295	1.696983	4.095475	0.348799	21.02326	30.94893
229	6.764656	1.360739	3.321074	1.436779	3.624854	0.347434	16.85554	25.38566
233	6.972369	1.473215	3.30155	1.433693	3.362893	0.33482	16.87854	25.14394
237	6.49264	1.534322	3.211033	1.406838	3.15914	0.312681	16.11665	24.0743
241	5.978907	1.53103	3.021047	1.350113	2.818394	0.292464	14.99196	22.37288
245	8.003785	1.421317	2.486262	1.100466	2.476042	0.383547	15.87142	22.3446
249	8.127533	1.495895	2.180277	1.091655	2.525572	0.397478	15.81841	22.16863
253	7.799584	1.519421	2.144139	1.055091	2.322511	0.383379	15.22413	21.2908
257	7.466741	1.540018	2.309352	1.02154	2.139539	0.360154	14.83734	20.77474
261	7.341782	1.543098	2.235492	0.943693	1.82071	0.323328	14.2081	19.6381
265	7.2773	1.51256	2.160701	0.875619	1.545548	0.298736	13.67046	18.64862
269	5.454013	1.209219	1.737171	0.679783	0.972214	0.219475	10.27187	13.9347
273	6.042654	1.271636	1.901676	0.728018	1.004482	0.253219	11.20168	15.12626
277	6.195368	1.314906	1.995173	0.763235	0.900627	0.231442	11.40075	15.3046
281	1.43043	0.305378	0	0	0	0	1.735809	1.86529
285	1.723472	0.374814	0	0	0	0	2.098286	2.257182
289	1.719626	0.360234	0	0	0	0	2.07986	2.232628

Table N4M. Acid concentration in F1 (Train D)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	1.497393	0	0.261701	0	0.093251	0	1.852345	2.138187
4	3.442335	0.208531	0.592137	0	0.134209	0	4.377211	5.029408
8	6.827057	0.482516	1.666511	0	0.263265	0	9.239349	10.90612
12	9.526296	0.758667	2.324953	0.229439	0.405358	0	13.24471	15.85628
16	9.596741	1.135185	2.994484	0.437979	1.291985	0.096566	15.55294	20.05049
20	9.167947	1.312606	3.136885	0.494413	2.273859	0.271739	16.65745	22.64172
24	9.463276	1.560047	3.601024	0.755213	3.522072	0.508532	19.41016	27.68371
28	7.408189	1.439601	3.150149	0.675372	3.806816	0.592679	17.07281	25.30817
32	8.875665	1.934579	3.883473	0.929424	4.469107	0.634989	20.72724	30.68114
36	8.12383	1.856094	3.892993	1.006188	4.534606	0.69149	20.1052	30.23994
40	6.784059	1.695999	3.397935	0.942925	4.480739	0.724205	18.02586	27.66634
44	8.230085	2.033314	4.227389	1.195745	5.31965	0.78138	21.78756	33.35392
48	8.014085	1.777479	3.732897	1.097441	4.91949	0.747752	20.28914	30.84036
52	7.867619	1.688125	3.707379	1.127016	4.920472	0.748823	20.05944	30.58432
56	7.731394	1.625938	3.670596	1.219168	4.827991	0.723969	19.79906	30.22721
60	7.762391	1.555227	3.580885	1.109633	4.765665	0.717928	19.49173	29.65287
64	7.820405	1.571094	3.565164	1.116521	4.695614	0.699898	19.4687	29.53522
68	8.174772	1.598609	3.653654	1.141376	4.741814	0.7414	20.05162	30.3142
72	7.083548	1.380703	3.197828	0.985896	4.138971	0.620429	17.40737	26.32378
76	7.406706	1.432882	3.246858	1.025293	4.229376	0.624682	17.9658	27.07686
80	7.760933	1.44166	3.445906	1.050326	4.356359	0.628312	18.6835	28.10222
84	7.597991	1.361203	3.040436	1.063835	3.817952	0.577232	17.45865	25.93206
88	7.772903	1.450471	3.217363	1.03195	4.051835	0.619275	18.1438	27.05126
92	7.563501	1.434151	3.173012	1.026738	3.976024	0.615822	17.78925	26.56832
96	9.131192	1.646297	3.663579	1.184744	4.213024	0.610477	20.44931	30.05682
100	7.754441	1.3705	2.90137	0.989713	3.620911	0.576721	17.21366	25.31365
104	8.413174	1.44886	2.874714	1.062694	3.74259	0.573573	18.11561	26.42343
108	6.000162	1.547395	3.273349	1.316929	4.051752	0.414007	16.60359	25.60491
112	6.077242	1.212736	2.062329	0.978669	2.983992	0.532744	13.84771	20.54315
116	5.668559	1.160813	1.965589	0.953423	2.742888	0.490774	12.98205	19.25572
120	6.262444	1.194897	2.212802	0.976729	2.287756	0.507847	13.44248	19.45985
124	6.402525	1.133967	2.19108	0.928554	1.839934	0.442711	12.93877	18.31407
128	6.661422	1.117875	2.161199	0.891738	1.738232	0.872645	13.44311	19.16165
132	6.58518	1.078142	2.089386	0.86476	1.656445	0.396965	12.67088	17.64173
136	7.452331	1.150745	2.214323	0.892518	1.625596	0.416443	13.75196	18.85782
140	7.863861	1.197399	2.347261	0.922864	1.728394	0.426935	14.48671	19.85668
144	7.524248	1.155762	2.281672	0.894552	1.528738	0.387059	13.77203	18.79067
148	7.148862	1.041605	2.086068	0.790836	1.332583	0.354051	12.75401	17.24212
152	7.621085	1.056074	2.097541	0.811489	1.33081	0.344803	13.2618	17.77041
156	7.75305	1.074211	2.144304	0.826597	1.347759	0.343993	13.48991	18.07024
160	6.765254	1.01853	2.090191	0.793162	1.231191	0.339414	12.23774	16.59484
169	6.737828	1.060208	2.214583	0.801095	1.469259	0.52872	12.81169	17.76229
173	6.177093	1.058533	2.134336	0.782926	1.114358	0.388317	11.65556	15.9841

177	6.145113	1.092891	1.822438	0.809737	0.860388	0.329903	11.06047	14.86603
181	5.577958	1.067423	1.751684	0.668405	0.604548	0.331138	10.00116	13.34406
185	6.314989	1.211356	1.627096	0.705872	0.433058	0.252071	10.54444	13.61666
189	5.275194	1.161503	0.954773	0.5044	0.182316	0	8.078185	9.900468
193	6.340851	1.426306	0.708706	0.411302	0.383673	0	9.270837	11.1621
197	4.69228	1.223243	0.416511	0.161377	0	0	6.493411	7.452646
201	5.135338	1.410353	0.448497	0.179941	0	0	7.174129	8.251874
205	4.313594	1.250982	0.191468	0.160669	0	0	5.916713	6.727765
209	3.614127	1.137671	0	0	0	0	4.751798	5.232747
213	3.25955	1.047171	0	0	0	0	4.306722	4.749356
217	1.717347	0.970445	0	0	0.305977	0	2.993769	3.730493
221	1.541343	0.920219	0	0	0	0	2.461562	2.849432
225	1.337019	0.830652	0	0	0	0	2.167671	2.517743
229	1.19967	0.761658	0	0	0	0	1.961328	2.282302
233	1.183147	0.693437	0	0	0	0	1.876584	2.168884
237	1.04031	0.654464	0	0	0	0	1.694775	1.970583
241	0.978595	0.608156	0	0	0	0	1.586751	1.843054
245	0.949182	0.592249	0	0	0	0	1.54143	1.791026
249	1.3336	0.520216	0	0	0.252849	0	2.106665	2.597042
253	1.234333	0.468972	0	0	0	0	1.703305	1.901349
257	1.130074	0.416409	0	0	0	0	1.546483	1.722359
261	0.991148	0.393452	0	0	0	0	1.3846	1.550716
265	0.959666	0.351883	0	0	0	0	1.311548	1.460175
269	0.880096	0.300983	0	0	0	0	1.181079	1.308256
273	0	0	0	0	0	0	0	0
277	0	0	0	0	0	0	0	0
281	0	0	0	0	0	0	0	0
285	0	0	0	0	0	0	0	0
289	0	0	0	0	0	0	0	0

Table N4N. Acid concentration in F2 (Train D)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	1.907947	0.131081	0.51624	0	0.58454	0	3.139808	4.186774
4	6.83717	0.524826	1.57486	0.080475	0.366914	0	9.384245	11.18857
8	8.556942	0.747747	2.50691	0.272503	0.580256	0	12.66436	15.62558
12	8.889734	0.951925	3.280795	0.426986	0.997608	0	14.54705	18.72861
16	8.327539	1.105037	3.499405	0.535143	1.698506	0.089425	15.25505	20.61082
20	7.686779	1.320326	3.625687	0.662872	2.509122	0.1926	15.99739	22.64
24	7.760101	1.504029	3.709119	0.718888	2.80832	0.235052	16.73551	23.93646
28	7.060048	1.612655	3.636322	0.828329	3.665459	0.377336	17.18015	25.56235
32	7.199981	1.71949	3.69173	0.881753	4.047655	0.453108	17.99372	27.00852
36	7.654477	1.89746	3.963924	0.959645	4.228419	0.466344	19.17027	28.73287
40	8.005634	1.994192	4.113659	1.051397	4.877746	0.564114	20.60674	31.21176
44	7.962313	1.959746	4.121635	1.123213	5.163057	0.623405	20.95337	31.99139
48	8.514247	1.851546	3.837732	1.119332	5.513173	0.752951	21.58898	32.90759
52	10.83816	2.192019	4.649085	1.404715	6.57401	0.873675	26.53167	40.10868
56	8.381059	1.747099	3.802324	1.156773	5.599776	0.768503	21.45553	32.85065
60	8.394957	1.710924	3.849632	1.197534	5.688127	0.778094	21.61927	33.17588
64	12.01164	2.247899	4.790094	1.554669	6.686861	0.877659	28.16882	42.13238
68	8.853041	1.744393	3.888649	1.257305	5.824423	0.795109	22.36292	34.18236
72	8.238232	1.62601	3.638299	1.19245	5.446396	0.747548	20.88893	31.96061
76	9.028061	1.774846	3.960212	1.318723	5.924877	0.884964	22.89168	35.04553
80	11.23392	2.092899	4.321391	1.480937	6.403763	0.903265	26.43617	39.66321
84	8.623585	1.688269	3.799263	1.281095	5.665624	0.793167	21.851	33.43296
88	9.084358	1.748605	3.867067	1.31474	5.714357	0.805819	22.53494	34.28851
92	11.06389	1.834551	3.899605	1.285124	5.233734	0.964249	24.28115	35.74298
96	11.39212	1.905119	4.10397	1.291386	5.360614	0.972318	25.02552	36.81275
100	9.442577	1.820125	4.001438	1.361255	5.765431	0.80608	23.19691	35.17316
104	5.130957	1.504092	2.105619	1.444437	5.695329	0.818404	16.69884	27.21598
108	3.797108	1.620926	2.59963	1.556744	5.184241	0.511225	15.26987	25.37229
112	6.694222	1.825547	2.881596	1.794544	5.982767	0.816435	19.99511	31.82159
116	5.209939	1.470285	2.384228	1.433742	4.988964	0.772009	16.25917	26.1374
120	7.302167	1.693732	3.014782	1.724791	5.660199	0.882522	20.27819	31.81367
124	9.545124	1.735482	3.423628	1.699286	4.829621	0.817592	22.05073	32.90413
128	10.02062	1.634894	3.258461	1.550324	4.210479	0.785738	21.46052	31.31845
132	11.60147	1.632335	3.401341	1.593686	4.39886	0.820067	23.44776	33.68909
136	11.52738	1.631237	3.301981	1.453066	3.530597	0.70591	22.15017	31.12644
140	13.38456	1.753068	3.750719	1.623354	4.00521	0.740668	25.25758	35.30858
144	12.47479	1.606625	3.479886	1.451928	3.274896	0.629305	22.91743	31.64345
148	10.92694	1.429259	3.170024	1.294392	2.822569	0.536827	20.18001	27.87286
152	11.73728	1.498384	3.384016	1.361237	2.930829	0.558555	21.4703	29.54685
156	13.74025	1.63558	3.63982	1.428154	2.968991	0.558702	23.9715	32.39009
160	12.45606	1.517844	0	1.411605	3.008437	0.583959	18.9779	24.83366
169	16.99624	1.593091	4.127863	1.612949	5.250659	0.790648	30.37145	42.00816
173	14.49298	1.42594	3.520542	1.322315	4.685533	0.769314	26.21662	36.45629

177	15.78248	1.509724	3.739199	1.427546	4.78608	0.812599	28.05762	38.74338
181	15.27325	1.501451	3.618608	1.349475	4.758244	0.796853	27.29788	37.77469
185	17.14513	1.692295	3.995542	1.443712	4.838561	0.819975	29.93522	40.95954
189	15.14729	1.542381	3.706717	1.343463	4.64922	0.803266	27.19234	37.63388
193	9.618156	1.265895	3.389543	1.319178	2.174622	0.460738	18.22813	25.24426
197	9.510341	1.243952	3.336213	1.277724	1.948166	0.44347	17.75987	24.42809
201	10.90674	1.421483	3.749114	1.42264	1.996343	0.446704	19.94302	27.16629
205	11.75646	1.414832	3.555262	1.313423	1.83182	0.506439	20.37823	27.25799
209	11.05775	1.455576	3.531775	1.2647	1.42535	0.437472	19.17262	25.49027
213	9.866383	1.414146	3.285706	1.220058	1.179566	0.367234	17.33309	23.07082
217	5.77109	1.320655	2.966131	1.151945	0.918622	0.272673	12.40112	17.41603
221	5.954203	1.338181	2.830418	1.095586	0.840876	0.271338	12.3306	17.12091
225	5.905142	1.346264	2.682528	1.065051	0.718962	0.241425	11.95937	16.45448
229	6.046916	1.364203	2.507184	0.989675	0.604249	0.224664	11.73689	15.90419
233	6.024103	1.390677	2.348277	0.97484	0.466904	0.184213	11.38901	15.24633
237	6.258332	1.421663	2.363636	1.003783	0.538946	0.198914	11.78527	15.78776
241	5.981012	1.371505	2.061682	0.876426	0.370205	0.166628	10.82746	14.25993
245	6.184553	1.351185	1.906564	0.825546	0.308922	0.150523	10.72729	13.91057
249	9.693891	1.360463	1.597339	0.679631	0.280249	0	13.61157	16.23966
253	9.95825	1.431784	1.353477	0.620517	0.190498	0	13.55453	15.89055
257	9.878054	1.523786	1.153004	0.55657	0	0	13.11141	15.08212
261	9.37712	1.515119	1.211759	0.245826	0	0	12.34983	14.07413
265	9.082265	1.586809	0.770423	0.40804	0	0	11.84754	13.43831
269	8.127951	1.487539	0.512275	0.20235	0	0	10.33011	11.50827
273	4.998209	0.989071	0.190267	0	0	0	6.177547	6.731544
277	4.852735	1.066702	0.206053	0	0	0	6.125491	6.723124
281	3.45299	0.820086	0	0	0	0	4.273076	4.620473
285	4.368964	0.577383	0.659157	0	0	0	5.605504	6.317047
289	0	0	0	0	0	0	0	0

Table N4O. Acid concentration in F3 (Train D)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	3.531207	0.242731	0.618089	0	0.191747	0	4.583774	5.330339
4	7.877992	0.568248	1.539413	0.099588	0.262199	0	10.34744	12.05119
8	8.418186	0.837087	3.020804	0.361748	0.819982	0	13.45781	17.15742
12	7.838559	1.083224	3.558652	0.470846	1.453172	0	14.40445	19.36428
16	7.495522	1.48251	4.062976	0.722412	2.474726	0.090389	16.32853	23.24333
20	7.022768	1.90019	4.266506	0.84874	3.057124	0.160975	17.2563	25.3131
24	6.969585	2.333835	4.233216	0.971929	3.940211	0.309362	18.75814	28.20878
28	6.955234	2.400091	4.014793	1.010195	4.5981	0.458152	19.43657	29.67785
32	6.714612	2.293145	3.652851	1.038124	4.871795	0.57127	19.1418	29.53607
36	8.595458	2.97649	4.69516	1.349839	5.773336	0.613236	24.00352	36.72236
40	7.704143	2.578066	4.027459	1.217107	5.277388	0.658017	21.46218	32.94276
44	8.126458	2.579419	4.187867	1.362792	6.009734	0.750591	23.01686	35.63932
48	9.897971	2.425456	4.21258	1.480505	6.749128	0.908149	25.67379	39.33782
52	9.247762	2.025504	4.049761	1.543081	7.052565	1.031408	24.95008	38.85983
56	8.415006	1.851968	3.901041	1.498994	6.771054	0.989072	23.42714	36.76606
60	8.147556	1.714763	3.761802	1.494148	6.622137	0.990917	22.73132	35.75242
64	11.00975	2.141195	4.780112	1.865841	7.406837	1.028191	28.23192	43.37761
68	9.433852	2.019805	4.148063	1.647872	6.813171	0.96184	25.0246	38.75783
72	9.041398	1.689979	3.916814	1.567216	6.456744	0.896358	23.56851	36.46611
76	8.760871	1.695489	3.982821	1.619167	6.693727	0.915767	23.66784	36.93849
80	9.359238	1.778235	4.208449	1.694806	7.407232	1.142226	25.59019	40.15883
84	8.456518	1.622124	3.884868	1.539597	6.298872	0.895853	22.69783	35.34903
88	8.433119	1.597362	3.791716	1.488877	6.083255	0.875148	22.26948	34.54258
92	0.255166	1.695443	4.015208	1.613686	6.133713	0.872283	14.5855	27.21537
96	8.392419	1.554609	3.774623	1.500826	5.955833	0.837101	22.01541	34.0875
100	8.897235	1.6249	3.91869	1.547917	5.630986	0.797995	22.41772	34.26998
104	8.723324	1.570214	3.976428	1.627392	6.098789	0.799221	22.79537	35.24019
108	7.078973	1.714665	4.617982	1.873361	5.959519	0.507356	21.75185	34.43592
112	9.479122	1.636093	4.110146	1.828509	7.077444	0.937526	25.06884	39.03305
116	9.30897	1.595237	3.825909	1.680458	5.907904	0.82529	23.14377	35.36854
120	12.72275	1.805214	4.105907	1.935972	6.495012	0.86531	27.93016	41.35344
124	12.42353	1.479658	3.202543	1.561255	5.523151	0.781518	24.97165	36.1373
128	16.3321	1.636921	3.424086	1.695161	5.595414	0.728454	29.41214	40.93982
132	15.82021	1.46153	3.043187	1.512239	5.312701	0.716515	27.86638	38.56703
136	17.49392	1.482788	3.127409	1.623817	5.80198	0.767168	30.29708	41.75377
140	16.82033	1.405845	2.863334	1.371935	4.034171	0.485899	26.98152	35.76019
144	18.79376	1.526499	3.145029	1.559059	4.734497	0.560327	30.31917	40.35866
148	17.09977	1.387812	2.551346	1.369824	4.494845	0.585122	27.48872	36.64959
152	15.13567	1.23418	2.295307	1.23724	3.968462	0.535351	24.40621	32.57603
156	19.41866	1.461262	2.640579	1.472229	4.520919	0.552297	30.06595	39.40486
160	18.25232	1.252103	2.397104	1.299816	4.247549	0.554259	28.00315	36.63351
169	16.05879	1.181021	2.524494	1.209401	3.591484	0.501823	25.06701	32.90798
173	17.31117	1.228955	2.676278	1.270927	3.995906	0.561234	27.04447	35.57392

177	17.957	1.236591	2.745661	1.301386	4.09766	0.574658	27.91296	36.64793
181	17.92041	1.223699	2.781989	1.317591	4.123331	0.581295	27.94832	36.7537
185	18.93171	1.27778	2.924929	1.379876	4.324381	0.612427	29.4511	38.69029
189	19.3696	1.354986	3.326033	1.650215	4.661513	0.611592	30.97394	41.13598
193	14.59842	1.044793	2.587225	1.243847	3.208898	0.447575	23.13076	30.51461
197	13.35916	1.045621	2.609134	1.239013	3.191312	0.471268	21.91551	29.3192
201	16.68788	1.267597	3.089314	1.544382	3.769447	0.453973	26.81259	35.52856
205	12.5235	1.073688	2.598448	1.239309	3.014477	0.435103	20.88453	28.05951
209	12.02832	1.088913	2.544458	1.189543	2.765093	0.421929	20.03826	26.85291
213	11.5696	1.108813	2.509391	1.145454	2.508939	0.402988	19.24519	25.70591
217	6.051747	1.019631	2.40071	1.050523	1.738601	0.245108	12.50632	17.74823
221	6.01055	1.033895	2.365602	1.029928	1.778375	0.260389	12.47874	17.74384
225	5.725128	1.047414	2.331622	0.990289	1.613722	0.234138	11.94231	16.94503
229	5.532503	1.047663	2.331066	0.92981	1.450261	0.235443	11.52675	16.30031
233	5.192912	1.028539	2.376952	0.882088	1.222478	0.214254	10.91722	15.40209
237	5.122359	1.010879	2.207412	0.835122	1.124355	0.201961	10.50209	14.69716
241	4.663227	1.003194	2.243239	0.766962	0.997417	0.265407	9.939445	14.03379
245	4.688779	1.018143	2.260682	0.760053	0.884257	0.175111	9.787025	13.66469
249	7.234409	0.966476	1.955251	0.597689	0.785343	0.229146	11.76831	15.22109
253	7.200591	0.93894	1.906111	0.590996	0.735766	0.231007	11.60341	14.95293
257	7.388653	0.907719	1.751457	0.538955	0.631702	0	11.21849	14.01106
261	6.616484	0.889933	1.702238	0.542774	0.550938	0	10.30237	12.9691
265	7.858358	1.019553	1.921909	0.664342	0.75719	0.246755	12.46811	15.97193
269	6.38321	0.842553	1.473066	0.471617	0.430214	0	9.600659	11.89125
273	5.685902	0.792874	1.331532	0.403103	0.285178	0	8.49859	10.44995
277	5.610137	0.767632	1.247457	0.414809	0.263194	0	8.303229	10.17172
281	5.608777	0.760721	1.166481	0.262657	0.221577	0	8.020213	9.645102
285	8.416257	1.097584	1.736017	0.626627	0.475814	0	12.3523	15.12776
289	4.259711	0.569154	0.672487	0.19713	0.182443	0	5.880925	6.973721

Table N4P. Acid concentration in F4 (Train D)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	Aceq (g/L)
0	3.184414	0.219485	0.54211	0	0.172479	0	4.118487	4.780729
4	6.350474	0.401702	0	0	0.164636	0	6.916812	7.267134
8	8.813653	0.66591	1.953681	0.217514	0.42202	0	12.07278	14.38962
12	9.856301	1.034702	2.507359	0.34262	0.932232	0	14.67321	18.19728
16	9.966193	1.300023	2.866843	0.444818	1.559736	0.175528	16.31314	21.17736
20	10.06833	1.559196	3.026559	0.566225	2.358702	0.340297	17.91931	24.16833
24	9.822044	1.686262	2.927556	0.695997	2.831421	0.462502	18.42578	25.42868
28	9.5011	1.817016	2.99288	0.670073	3.050575	0.523385	18.55503	25.94237
32	9.979326	1.925043	2.995927	0.827817	4.298388	0.872234	20.89873	30.23068
36	9.691472	2.045443	3.023438	0.822868	4.0985	0.807877	20.4896	29.59592
40	11.98368	2.553375	3.691856	1.024714	4.761335	0.871039	24.886	35.64897
44	10.80085	2.370758	3.487857	0.996781	4.552049	0.888378	23.09667	33.40903
48	14.36399	2.644458	3.940355	1.169457	5.396857	1.013107	28.52822	40.48917
52	14.23894	2.563644	4.268951	1.28803	5.905883	1.137133	29.40258	42.36286
56	12.08344	2.125078	3.981619	1.222694	5.49332	1.07726	25.98341	37.98191
60	12.76452	2.21215	4.226283	1.323077	5.894304	1.144353	27.56469	40.37419
64	13.53809	2.263371	4.395857	1.386863	6.166841	1.209291	28.96032	42.3393
68	14.33768	2.409891	4.747065	1.495497	6.285547	1.111708	30.38739	44.18633
72	11.52974	1.890713	3.85351	1.211977	5.097453	0.930601	24.51399	35.71436
76	9.451533	1.60794	3.406509	1.075354	4.757467	0.857984	21.15679	31.34554
80	11.46719	1.86921	3.977206	1.264165	5.320162	0.939686	24.83762	36.41319
84	11.20084	1.849394	4.02977	1.27172	5.311854	0.99057	24.65415	36.31706
88	11.70559	1.899324	4.084791	1.313861	5.414368	1.229997	25.64793	37.80523
92	10.06342	2.013656	2.296098	0.487635	2.09418	0.284168	17.23916	22.74139
96	11.33982	1.994981	2.471365	0.69472	2.048129	0.238602	18.78762	24.49222
100	13.43216	1.916709	4.203714	1.3453	5.49671	0.969983	27.36457	39.42096
104	14.08925	1.907679	4.415422	1.427415	5.813005	1.028772	28.68154	41.36799
108	10.33139	2.035339	4.945557	1.591074	5.752553	0.668211	25.32413	38.08953
112	16.03435	2.173756	4.849242	1.55622	5.684911	0.989717	31.28819	44.32817
116	13.67219	1.877673	4.44208	1.647862	5.540885	0.958732	28.13942	40.6581
120	15.2758	1.975542	4.820648	1.684379	7.073938	1.178423	32.00873	46.77423
124	13.24406	1.669119	4.059592	1.405345	5.49415	0.977072	26.84934	38.76038
128	13.74153	1.663005	4.037104	1.424737	5.41316	1.102818	27.38236	39.35652
132	14.2915	1.615395	3.560584	1.374257	5.259492	0.887013	26.98824	38.13811
136	15.782	1.706946	4.030873	1.43194	5.400393	0.93189	29.28404	41.06277
140	14.46821	1.597207	3.811439	1.38656	5.124579	0.778205	27.1662	38.22253
144	13.45719	1.493199	3.570074	1.292637	4.723637	0.749095	25.28583	35.5774
148	14.22914	1.534872	3.655707	1.312397	4.751121	0.736194	26.21943	36.62168
152	15.26177	1.600374	3.785842	1.367387	5.015036	0.776577	27.80699	38.7105
156	15.86433	1.635356	3.574326	1.39238	4.71759	0.70862	27.8926	38.28519
160	13.75871	1.495774	3.439341	1.263921	4.49308	0.717353	25.16818	35.05781
169	12.15951	1.565253	4.190441	1.697608	3.294022	0.655945	23.56278	33.04947
173	10.8626	1.381824	3.612633	1.439647	3.009995	0.687222	20.99392	29.49219

177	11.10264	1.375179	3.486106	1.305243	2.71152	0.657318	20.63801	28.56632
181	11.37994	1.391861	3.472429	1.338951	2.769857	0.609581	20.96261	28.92476
185	11.4412	1.418676	3.471917	1.343087	2.861674	0.649047	21.1856	29.30791
189	11.46829	1.446934	3.47144	1.336126	2.812228	0.598329	21.13335	29.14735
193	14.11225	1.339079	3.383984	1.264763	3.70954	0.568572	24.37819	33.14737
197	12.82569	1.288253	3.278364	1.260352	3.631656	0.545902	22.83022	31.38793
201	16.10384	1.554918	3.915136	1.599234	4.737295	0.682634	28.59306	39.37166
205	14.06371	1.471374	3.646256	1.465304	4.10546	0.585128	25.33723	34.97395
209	11.8585	1.338152	3.202546	1.299831	3.774844	0.584317	22.05819	30.81777
213	10.91	1.322264	3.077547	1.259199	3.493052	0.555844	20.6179	28.90895
217	6.070074	1.164239	2.670671	1.161217	2.860532	0.442335	14.36907	21.40018
221	5.187303	1.237609	2.577574	1.116458	2.488486	0.343775	12.95121	19.38976
225	5.982694	1.376318	2.620595	1.103442	2.510645	0.407852	14.00155	20.61777
229	5.226329	1.325931	2.546999	1.048489	2.104181	0.343862	12.59579	18.57645
233	5.107144	1.34242	2.669176	1.059304	1.796118	0.296825	12.27099	17.9686
237	5.073586	1.357042	2.616253	1.031138	1.682761	0.289339	12.05012	17.56045
241	5.107402	1.356173	2.652282	1.006735	1.419855	0.268979	11.81143	17.01876
245	5.255283	1.361782	2.678373	1.003731	1.183448	0.250069	11.73269	16.68248
249	7.945733	1.312316	2.400086	0.864733	1.283231	0.520858	14.32696	19.36499
253	7.978822	1.221878	2.261732	0.81196	1.130424	0.451254	13.85607	18.46354
257	8.050273	1.217581	2.166264	0.771881	0.903355	0.393383	13.50274	17.69223
261	7.925124	1.218936	2.129625	0.767325	0.814603	0.36572	13.22133	17.2532
265	8.918988	1.28431	2.120991	0.786583	0.724769	0.335646	14.17129	18.11085
269	8.537217	1.215678	1.859264	0.699457	0.589217	0.311142	13.21197	16.68364
273	5.914082	0.886236	1.33282	0.460164	0.282385	0	8.875687	10.91649
277	7.719136	1.134256	1.559152	0.61404	0.308732	0	11.33532	13.81035
281	7.027659	1.066132	1.420336	0.570742	0.266771	0	10.35164	12.61501
285	5.717881	0.845999	1.097501	0.453098	0.273522	0	8.388001	10.22967
289	6.323642	0.923861	1.125132	0.463411	0.198944	0	9.03499	10.85895

Table N5A. Total acid equivalent for ash and lime pretreated biomass

Time (weeks)	Acids Ash (mL)	Deviation (mL)	Acids Lime (mL)	Deviation (mL)
0	7.3	0.141421	8.55	0.212132
1	4.5	0.424264	3.6	0.141421
2	4.5	0.424264	2.25	0.070711
3	4.35	0.919239	2.45	0.070711
4	3.5	0.707107	1.45	0.070711
6	2.75	0.212132	1.1	0.282843

Table N5B. Total lignin in ash- and lime-treated poplar wood

Time (weeks)	Ash lignin (%)	Deviation (%)	Lime lignin (%)	Deviation (%)
0	22.05	0.17	23.60	1.01
1	20.63	0.38	21.04	0.27
2	19.63	1.11	19.21	0.08
3	20.40	0.96	21.31	0.42
4	19.09	1.80	20.14	0.77
6	18.78	0.80	19.13	0.02

Table N5C. Total sugar yield in ash and lime pretreated poplar wood

Time (weeks)	Ash yield (mg equiv. glucose/g dry biomass)	Deviation (mg equiv. glucose/g dry biomass)	Lime yield (mg equiv. glucose/g dry biomass)	Deviation (mg equiv. glucose/g dry biomass)
0	104.54	5.94	222.61	58.29
1	287.74	5.94	529.22	96.08
2	250.72	11.87	592.07	26.63
3	296.52	0.00	593.59	49.66
4	387.35	134.94	507.59	76.29
6	464.83	100.93	609.88	9.36

Table N7A. Fermentation data for Experiment 1 under mesophilic conditions F1 ($CaCO_3$)

Time (d)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	% C2
0	0	0	0	0	0	0	0.267597	0.336107	0.603704	0
2	0.812573	0	0	0	0	0	0	0	0.812573	1
4	1.385533	0	0	0	0	0	0	0	1.385533	1
6	1.781487	0	0	0.452045	0	0	0	0	2.233532	0.79761
8	3.403215	0	0	0.782556	0	0	0	0	4.185772	0.813044
10	3.450766	0	0	0.776295	0	0	0	0	4.227062	0.816351
12	1.662649	0	0	1.540517	0	0.167537	1.567669	0	4.938373	0.33668
14	3.218688	0	0	1.614582	0	0.201867	1.88347	0	6.918608	0.465222
16	4.938557	0.856153	0	1.93793	0	0.249147	2.188829	0	10.17062	0.485571
18	6.844259	0.823033	0	1.784607	0	0.273147	2.649149	0.409004	12.7832	0.535411
20	8.184876	0.839544	0	1.818527	0	0.257002	2.563728	0.223903	13.88758	0.589367

F2 (NH_4HCO_3 step)

Time (d)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	% C2
0	0	0	0	0	0	0	0.32145	0	0.32145	0
2	2.029208	0	0	0.36224	0	0	0.191521	0	2.582968	0.785611
4	3.255409	0	0	0.420526	0	0	0	0	3.675935	0.8856
6	4.838801	0	0	0.494992	0	0	0	0	5.333792	0.907197
8	6.713225	0	0	0.427975	0	0	0	0	7.1412	0.94007
10	10.06747	0.28299	0	0.501192	0	0	0	0	10.85165	0.927736
12	11.13824	0.26761	0	0.602235	0	0	0	0	12.00809	0.927562
14	13.6939	0.28235	0	0.767777	0	0	0	0	14.74403	0.928776
16	14.12594	0.25850	0.17283	0.855148	0.161239	0	0	0	15.57366	0.90704
18	13.44906	0	0.151737	0.699293	0.134981	0	0.413351	0	14.84843	0.905757
20	13.16958	0	0.162736	0.614376	0	0	0.227556	0	14.17425	0.92912

F3 (NH_4HCO_3 total)

Time (d)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	% C2
0	0	0	0	0	0	0	0	0	0	0
2	1.949777	0	0	0	0	0	0	0	1.949777	1
4	4.137866	0	0	0	0	0	0	0	4.137866	1
6	4.879128	0	0	0	0	0	0	0	4.879128	1
8	7.335663	0	0	0	0	0	0	0	7.335663	1
10	7.436798	0	0	0	0	0	0	0	7.436798	1
12	8.651084	0	0	0	0	0	0	0	8.651084	1
14	8.369459	0	0	0	0	0	0	0	8.369459	1
16	20.27506	0	0	0.196672	0	0	0	0	20.47173	0.990393
18	19.65833	0	0	2.887142	0	0	0	0	22.54547	0.871941
20	25.90389	0	0.193803	3.580934	0	0	0	0	29.67863	0.872813

Table N7B. Fermentation data for Experiment 2a under mesophilic conditions F1 ($CaCO_3$)

Time (d)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	% C2
0	8.541096	0.466445	0	1.499479	0	0.184338	1.864317	0.065677	12.62135	0.67671797
2	9.362513	0.440328	0	1.465438	0	0.177203	1.756283	0.05762	13.25938	0.70610466
4	11.18322	0.47338	0	1.647178	0	0.187144	1.856041	0.059959	15.40692	0.72585687
6	12.49163	0.481305	0	1.730665	0	0.189863	1.868418	0.058586	16.82047	0.74264465
8	13.64693	0.486295	0	1.738584	0	0.186165	1.818413	0.05629	17.93268	0.76100912
10	14.92338	0.50475	0	1.774963	0	0.190347	1.841756	0.055352	19.29054	0.77361099
12	15.06088	0.494611	0	1.722269	0	0.18068	1.74573	0.053391	19.25756	0.7820762
14	15.42957	0.502093	0	1.722297	0	0.178593	1.723351	0.052627	19.60853	0.78688045
16	18.14724	0.595826	0	2.022743	0	0.205419	1.978245	0.061504	23.01098	0.78863409
18	19.09431	0.612052	0	2.122875	0	0.222274	2.002763	0.060831	24.11511	0.79179874
20	20.82498	0.687388	0.056172	2.457502	0	0.226344	2.151416	0.063539	26.46734	0.78681797
22	21.5877	0.811015	0.057705	2.632428	0	0.232693	2.188737	0.065985	27.57626	0.7828363
24	21.43521	0.870032	0.056177	2.634348	0	0.22951	2.128919	0.063574	27.41777	0.7817999
26	21.98786	0.877338	0.058057	2.771733	0	0.237821	2.184744	0.068412	28.18596	0.78009955
28	22.76664	0.836051	0.058997	2.802655	0	0.239437	2.167858	0.068134	28.93977	0.78669038
30	23.91848	0.780482	0.062213	3.018305	0	0.250472	2.25396	0.064915	30.34882	0.78811873
32	24.31013	0.740706	0.063388	3.103445	0	0.253561	2.27393	0.066279	30.81144	0.78899692
34	23.89074	0.518955	0.065719	3.236057	0	0.257956	2.319804	0.068275	30.35751	0.78697965
36	24.3198	0.465732	0.067186	3.322226	0	0.257791	2.329724	0.063352	30.82581	0.78894274
38	25.10278	0.446096	0.069169	3.433116	0	0.260545	2.391704	0.066438	31.76985	0.7901448
40	25.75015	0.319132	0.071914	3.578566	0	0.265788	2.482119	0.065678	32.53335	0.79150019
44	19.77391	0.15309	0.060003	2.971661	0	0.201286	1.93761	0.0494	25.14696	0.78633398

F2 (NH_4HCO_3 step)

Time (d)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	% C2
0	7.870978	0	0.122311	0.139444	0.113569	0	0.155575	0	8.401876	0.93681195
2	11.04494	0.146304	0.146045	0.266569	0.135302	0	0.156978	0	11.89614	0.92844756
4	12.52366	0.250092	0.164049	0.312376	0.159988	0	0.14901	0	13.55917	0.92362994
6	13.67287	0.225666	0.181232	0.326746	0.177144	0	0.153343	0	14.737	0.92779187
8	15.11741	0.211151	0.200513	0.341599	0.197312	0	0.150273	0	16.21826	0.93212296
10	16.19406	0.205276	0.21013	0.331685	0.210422	0	0.154102	0	17.30567	0.93576585
12	17.35765	0.202722	0.214844	0.292313	0.22101	0	0.151108	0	18.43964	0.94132224
14	17.67319	0.201688	0.222732	0.302202	0.235795	0	0.152739	0	18.78834	0.94064648
16	18.91177	0.212714	0.235908	0.321681	0.259807	0	0.162968	0	20.10485	0.94065721
18	20.01279	0.217841	0.242674	0.341974	0.271711	0	0.161084	0	21.24808	0.94186368
20	20.84915	0.221814	0.251263	0.383053	0.286612	0	0.16302	0	22.15491	0.94106219
22	20.22688	0.212154	0.245952	0.399336	0.283584	0	0.156556	0	21.52447	0.93971587
24	21.09349	0.220032	0.262466	0.437886	0.307818	0	0.161835	0	22.48353	0.93817529
26	20.57579	0.234817	0.264073	0.501191	0.319887	0	0.191035	0	22.0868	0.93158794
28	20.43119	0.232539	0.274406	0.495919	0.331811	0	0.175732	0	21.9416	0.93116238
30	20.19003	0.248788	0.285468	0.545327	0.35365	0	0.179073	0	21.80233	0.92604892
32	20.71824	0.267993	0.303725	0.603988	0.385731	0	0.175635	0	22.45531	0.92264318
34	23.73183	0.307932	0.351316	1.239141	0.424773	0	0.163371	0	26.21837	0.90516066
36	20.90296	0.275533	0.327373	0.695022	0.422546	0	0.171266	0	22.7947	0.91700962
38	21.35023	0.290096	0.344086	0.736255	0.444826	0	0.178756	0	23.34425	0.91458201
40	21.66235	0.29949	0.359678	0.762813	0.461175	0	0.180961	0	23.72647	0.91300362
44	21.52105	0.303477	0.372126	0.822989	0.476361	0	0.166529	0	23.66253	0.90949906

F3 (NH_4HCO_3 total)

Time (d)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	% C2
0	14.05715	0.116677	0.131001	2.08353	0.070955	0	0.074446	0	16.53376	0.85020898
2	18.69	0.160303	0.164556	2.288379	0.078032	0	0.096463	0	21.47773	0.87020356
4	21.63809	0.184235	0.200001	2.35921	0.1281	0	0.104557	0	24.61419	0.87908992
6	22.81889	0.16978	0.215342	2.344771	0.144656	0	0.11033	0	25.80376	0.8843239
8	26.67111	0.203194	0.245682	2.416173	0.180469	0	0.117079	0	29.83371	0.89399253
10	28.68109	0.227048	0.260165	2.460633	0.198822	0	0.121322	0	31.94908	0.89771256
12	29.08371	0.230987	0.266427	2.330528	0.207623	0	0.122796	0	32.24207	0.90204225
14	28.30833	0.208892	0.264378	2.163912	0.204748	0	0.12148	0	31.27174	0.90523682
16	31.82478	0.235976	0.300621	2.315771	0.238714	0	0.136281	0	35.05214	0.90792679
18	33.58192	0.242927	0.318985	2.31613	0.262621	0	0.139635	0	36.86222	0.9110119
20	32.11166	0.239398	0.320874	2.245453	0.268727	0	0.1354	0	35.32152	0.9091247
22	31.61463	0.232695	0.318206	2.174047	0.268974	0	0.13289	0	34.74144	0.90999765
24	31.74703	0.24215	0.333732	2.215265	0.291669	0	0.134314	0	34.96416	0.90798776
26	31.29024	0.261265	0.348112	2.272167	0.328783	0	0.140465	0	34.64104	0.90327102
28	31.05184	0.285583	0.367164	2.275232	0.347533	0	0.135028	0	34.46238	0.90103585
30	30.9302	0.322757	0.394979	2.44484	0.395719	0	0.14118	0	34.62968	0.89317037
32	30.66288	0.366503	0.418361	2.646487	0.436079	0	0.136403	0	34.66672	0.884505
34	20.80787	0.265714	0.314477	0.644944	0.400523	0	0.172323	0	22.60585	0.92046391
36	31.94513	0.451791	0.467442	3.015755	0.523562	0	0.137888	0	36.54157	0.87421347
38	32.18019	0.488927	0.497748	3.187016	0.566029	0	0.137009	0	37.05692	0.86839896
40	31.71594	0.515404	0.517889	3.318582	0.597446	0	0.13511	0	36.80037	0.86183752
44	33.01818	0.670422	0.582306	3.726611	0.702818	0	0.133646	0	38.83398	0.85023936

Table N7C: Fermentation data for Experiment 2b under mesophilic conditions F1 ($CaCO_3$)

Time (d)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	% C2
0	1.087001	0	0	0.18323	0	0	0.228576	0	1.498807	0.72524427
2	1.479971	0	0	0.178558	0	0	0.205217	0	1.863746	0.79408384
4	2.502497	0	0	0.273689	0	0	0.230192	0	3.006378	0.83239585
6	4.615483	0.195613	0	0.487357	0	0	0.315615	0	5.614068	0.82212808
8	4.36325	0.211555	0	0.505346	0	0	0.234738	0	5.31489	0.82094834
10	4.709565	0.293662	0	0.578007	0	0	0.225356	0	5.806591	0.8110723
12	6.193668	0.393521	0	0.762638	0	0	0.268224	0	7.618051	0.81302525
14	8.156519	0.421783	0	0.960679	0	0	0.320868	0	9.859848	0.82724594
16	8.684564	0.36789	0	0.986067	0	0	0.324024	0	10.36255	0.83807244
18	7.580761	0.237164	0	0.823808	0	0	0.267105	0	8.908839	0.85092582
20	9.114721	0.167609	0	0.999159	0	0	0.317806	0	10.5993	0.8599365
22	8.231881	0.097949	0	0.867787	0	0	0.275165	0	9.472781	0.86900357
24	9.979856	0	0	1.028041	0	0	0.323828	0	11.33173	0.8807005
26	10.03704	0	0	1.039194	0	0	0.328097	0	11.40433	0.88010775
28	9.737698	0	0	1.023994	0	0	0.323341	0	11.08503	0.87845455
30	9.815772	0	0	1.045789	0	0	0.326267	0	11.18783	0.87736174
32	10.64468	0	0	1.065963	0	0	0.331674	0	12.04232	0.88393958
34	10.77284	0	0	1.060856	0	0	0.328812	0	12.16251	0.88574165
36	11.35017	0	0	1.095308	0	0	0.342579	0	12.78806	0.88756018
38	11.43543	0	0	1.093164	0	0	0.343941	0	12.87253	0.88835883
40	11.71728	0	0	1.123682	0	0	0.352969	0	13.19393	0.88808105

F2 (NH_4HCO_3 step)

Time (d)	**C2 (g/L)**	**C3 (g/L)**	**IC4 (g/L)**	**C4 (g/L)**	**IC5 (g/L)**	**C5 (g/L)**	**C6 (g/L)**	**C7 (g/L)**	**Total (g/L)**	**% C2**
0	1.357886	0	0	0	0	0	0	0	1.357886	1
2	2.547243	0.130235	0	0.158275	0	0	0	0	2.835753	0.89825973
4	5.162999	0.196959	0	0.175565	0	0	0	0	5.535523	0.93270301
6	5.711971	0.162382	0	0.157278	0.053613	0	0	0	6.085243	0.93865934
8	5.451213	0.119443	0.056964	0.122035	0.059793	0	0	0	5.809447	0.93833591
10	5.832841	0.111505	0.065925	0.115834	0.072474	0	0	0	6.19858	0.94099641
12	6.809009	0.101924	0.078395	0.102714	0.086453	0	0	0	7.178495	0.94852876
14	6.081402	0.088293	0.079446	0.090278	0.086079	0	0	0	6.425497	0.94644848
16	6.544638	0.079324	0.091248	0.085249	0.09717	0	0	0	6.897629	0.94882435
18	6.452143	0	0.092309	0.071039	0.098802	0	0	0	6.714294	0.96095641
20	6.462523	0	0.090246	0.057502	0.094998	0	0	0	6.705269	0.96379769
22	7.390729	0	0.095076	0.057431	0.098093	0	0	0	7.641329	0.96720472
24	8.675825	0	0.114464	0.089062	0.118008	0	0	0	8.997359	0.96426352
26	8.077154	0	0.114759	0.056228	0.118697	0	0	0	8.366838	0.96537711
28	7.947151	0	0.120488	0.058668	0.119822	0	0	0	8.246129	0.96374325
30	7.581305	0	0.12214	0.060996	0.121008	0	0	0	7.885449	0.96142971
32	7.982096	0	0.127773	0.064723	0.127288	0	0	0	8.301879	0.96148062
34	7.678936	0	0.13174	0.056742	0.130142	0	0	0	7.997559	0.96015993
36	7.857509	0	0.13441	0.058277	0.130449	0	0	0	8.180645	0.96049995
38	8.578227	0	0.14049	0.059132	0.13536	0	0	0	8.913209	0.96241741
40	8.965664	0	0.142924	0.06291	0.137003	0	0	0	9.308501	0.9631695

F3 (NH_4HCO_3 total)

Time (d)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	% C2
0	2.985945	0	0	0.443431	0	0	0	0	3.429376	0.8706964
2	3.618962	0	0	0.285596	0	0	0	0	3.904558	0.92685568
4	6.737434	0	0	0.317439	0	0	0	0	7.054873	0.9550043
6	7.235696	0	0	0.214178	0	0	0	0	7.449874	0.97125073
8	7.664469	0	0.056273	0.166702	0.047327	0	0	0	7.934772	0.96593443
10	8.201104	0	0.064194	0.158366	0.05834	0	0	0	8.482004	0.9668829
12	8.162204	0	0.070491	0.127178	0.06336	0	0	0	8.423232	0.96901085
14	8.05341	0	0.077293	0.104593	0.069835	0	0	0	8.305132	0.96969083
16	9.180809	0	0.091929	0.099839	0.085167	0	0	0	9.457744	0.97071868
18	9.156744	0	0.094011	0.076517	0.086767	0	0	0	9.414038	0.97266908
20	8.824432	0	0.094392	0	0.083557	0	0	0	9.002381	0.98023315
22	9.168361	0	0.101464	0	0.0842	0	0	0	9.354026	0.98015136
24	9.645929	0.077905	0.112596	0	0.094515	0	0	0	9.930946	0.9713002
26	9.805684	0.084141	0.122577	0	0.108916	0	0	0	10.12132	0.9688149
28	9.368331	0.08256	0.125594	0	0.107592	0	0	0	9.684077	0.9673953
30	9.163488	0.087957	0.13005	0	0.113915	0	0	0	9.49541	0.96504392
32	8.709627	0.075946	0.136045	0.061848	0.120255	0	0	0	9.103721	0.95671059
34	8.644797	0.082368	0.14177	0.075998	0.133201	0	0	0	9.078134	0.95226578
36	9.029772	0.090409	0.149633	0.086861	0.1442	0	0	0	9.500874	0.95041483
38	8.136543	0.088457	0.152515	0.09842	0.157338	0	0	0	8.633274	0.94246318
40	7.436351	0.08801	0.158308	0.107389	0.166919	0	0	0	7.956978	0.9345698

Table N7D. Fermentation data for Experiment 3 under mesophilic conditions F1 ($CaCO_3$)

Time (d)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	% C2
0	0.618194	0	0	0.066243	0	0	0.048533	0	0.73297	0.843409
2	1.686329	0	0	0.240475	0	0	0.055095	0	1.981899	0.850866
4	1.999394	0.346838	0	0.515332	0	0.056938	0.113211	0	3.031713	0.659493
6	2.149554	0.387563	0	1.46461	0	0.472895	1.628594	0	6.103216	0.3522
8	1.913231	0.254472	0	1.397461	0	0.482893	1.644593	0	5.692649	0.336088
10	2.900753	0.760175	0	1.375684	0	0.522317	1.773242	0	7.33217	0.39562
12	4.298824	1.538513	0	2.008352	0	0.705169	1.952837	0	10.5037	0.409268
14	4.830262	1.582009	0.074958	2.059945	0.053738	0.719612	1.932315	0	11.25284	0.429248
16	4.472878	1.366774	0.077777	1.939471	0.051013	0.757996	1.946936	0	10.61285	0.421459
18	2.866912	0.453171	0.085948	2.507513	0.054761	1.565805	4.535392	0.124539	12.19404	0.235108
20	2.68788	0.556137	0.084966	2.270349	0	1.477335	4.688379	0.160697	11.92574	0.225385
22	2.89133	0.586552	0.097675	2.211615	0	1.584134	4.816302	0.159122	12.34673	0.234178
24	3.68713	0.850276	0.106849	2.385502	0	1.6964	5.106631	0.163519	13.99631	0.263436
26	3.826076	1.132702	0.102644	2.135142	0	1.606114	4.885189	0.155893	13.84376	0.276376
28	3.207045	1.328808	0.089132	1.729827	0	1.404384	4.246749	0.132681	12.13863	0.264202
30	3.735331	1.510029	0.108114	1.937281	0	1.705125	5.107328	0.159914	14.26312	0.261887
32	4.175739	1.737392	0.115739	1.914543	0	1.729974	5.026004	0.154848	14.85424	0.281114

F2 (NH_4HCO_3 step)

Time (d)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	% C2
0	0.31577	0	0	0	0	0	0	0	0.31577	1
2	1.757271	0	0	0.103553	0	0	0	0	1.860824	0.944351
4	2.996896	0.841199	0	0.259971	0	0	0	0	4.098066	0.731295
6	4.518041	1.367295	0.062999	2.379529	0	0.774199	0.746556	0	9.848619	0.458749
8	4.770034	1.330181	0.064018	2.272132	0	0.764012	0.740258	0	9.940636	0.479852
10	5.6404	3.191379	0.074803	2.839561	0	0.789254	0.767035	0	13.30243	0.424013
12	8.624779	4.540095	0.088376	3.42825	0	0.944708	0.896291	0	18.5225	0.465638
14	9.547875	5.395343	0.09478	3.375344	0.064343	0.985594	0.944631	0	20.40791	0.467852
16	9.638167	5.529181	0.097419	3.17593	0.069855	0.957023	0.914742	0	20.38232	0.472869
18	9.897409	5.627059	0.11737	3.182678	0.093235	0.980236	0.939732	0	20.83772	0.474976
20	9.445699	5.235792	0.121304	2.947717	0.083587	0.935139	0.894903	0	19.66414	0.480351
22	10.32433	5.65134	0.131132	3.098336	0.080603	1.025527	0.984427	0	21.29569	0.484808
24	11.08291	5.395687	0.130612	3.300801	0.057713	0.990098	0.939511	0	21.89733	0.50613
26	13.3462	5.300368	0.149358	3.349069	0.061482	0.936334	0.877214	0	24.02002	0.555628
28	14.28737	5.297374	0.172982	5.070357	0.088118	0.921226	0.865185	0	26.70262	0.535055
30	14.85932	5.157403	0.200588	5.837458	0.151955	0.922959	0.875702	0	28.00539	0.530588
32	15.22824	4.916268	0.221821	6.10623	0.195846	0.907473	0.864502	0	28.44038	0.535444

F3 (NH_4HCO_3 total)

Time (d)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	% C2
0	0.315851	0	0	0	0	0	0	0	0.315851	1
2	1.403601	0.077807	0	0.188483	0	0	0	0	1.669891	0.840535
4	2.72191	0.323587	0	0.200104	0	0	0	0	3.2456	0.838646
6	4.003207	0.673652	0	0.560057	0	0.092447	0.082042	0	5.411404	0.739772
8	4.092399	0.606285	0	0.542284	0	0.108867	0.099606	0	5.449441	0.750976
10	4.428165	0.675599	0	2.488206	0	0.450924	0.854222	0	8.897115	0.497708
12	4.876958	1.146536	0.117928	3.513341	0.063425	1.15324	4.485603	0.089316	15.44635	0.315735
14	6.254916	1.621271	0.183568	4.008484	0.087059	1.540858	5.176503	0.131673	19.00433	0.329131
16	7.389158	1.466737	0.19784	3.790928	0.070503	1.485642	5.028223	0.130428	19.55946	0.377779
18	8.642023	1.236151	0.207495	3.665366	0.066824	1.43276	4.893137	0.12325	20.26701	0.426408
20	8.786842	1.028972	0.201226	3.373625	0.057402	1.334227	4.576339	0.116132	19.47477	0.451191
22	9.853925	1.057792	0.216934	3.571624	0.059374	1.422855	4.895601	0.12985	21.20796	0.464633
24	9.008038	0.957254	0.215088	4.69094	0.06967	1.282153	4.432493	0.112344	20.76798	0.433746
26	11.07834	1.056587	0.283707	6.094251	0.163253	1.279917	4.457209	0.114062	24.52732	0.451673
28	12.79965	1.144844	0.339318	7.952601	0.265881	1.334389	4.684203	0.124072	28.64496	0.446838
30	12.9084	1.014578	0.348295	8.068168	0.291136	1.292292	4.544677	0.121584	28.58913	0.451514
32	13.36725	0.910813	0.372041	8.532679	0.33164	1.322432	4.682675	0.127293	29.64683	0.450883

VITA

Frank Kwesi Agbogbo received his Bachelor of Science degree in chemical engineering in June 2000 from the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. He worked as a teaching assistant at the same university for 1 year and joined the Department of Chemical Engineering at Texas A&M University in the fall of 2001. He studied under the guidance of Dr. Mark T. Holtzapple for 4 years in pursuit of his Ph.D. in chemical engineering. Frank Kwesi Agbogbo's permanent address is House No. N/3 236, Ashaiman, Ghana.